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A TRANSMISSIBLE MOSAIC DISEASE OF LETTUCE

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During January, 1920, Romaine lettuce (variety Paris White Cos) in a field of several acres at Sanford, Fla., developed a condition very suggestive of a transmissible mosaic disease. The first symptom of disease was a yellowish discoloration along the smaller veins of the younger expanding leaves. This symptom was usually evident for only a few days, giving way to a general yellowish, discolored appearance of the whole plant. All gradations of discoloration occurred, from very marked to conditions not distinguishable with certainty from normal. Close examination usually revealed irregular blotches of an approximately normal green color, which were usually located along the larger leaf veins. The blotching varied from a few barely perceptible green areas on a yellowish leaf to numerous pronounced green spots giving a marked mottled appearance to an occasional plant (Pl. 87, A). The leaves of diseased plants generally seemed to be rather more wrinkled than those of normal plants. Where plants became diseased only after reaching considerable size, the older leaves, which were fully expanded on the first appearance of disease symptoms, frequently continued to appear perfectly normal, while all younger leaves developed the disease symptoms.

At the same time head lettuce (variety Big Boston) in a neighboring field developed a similar diseased condition. The general yellowish, discolored appearance of whole plants was frequently pronounced, but in most cases the blotching was less marked than in the Romaine lettuce, and a decided mottled appearance was never observed.

In general, diseased plants made a stunted growth. In severe cases the plants were decidedly undersized, and occasionally the leaves formed only a rosette, with no indications of a folding together of the tips to form a head. Usually loose heads of poor quality were formed, although all gradations of development, including occasional heads of practically normal size and hardness, occurred. Often plants that showed marked discoloration, mottling, and stunting soon after becoming diseased would later seem to recover in part and to make a more or less normal growth with only slight discoloration and mottling.

Attempts to islolate fungi or bacteria from the apparently healthy plants were unsuccessful, at least in so far as it has not been possible to isolate any organisms capable of producing the disease on reinoculation. Furthermore, examination of the etiolated areas of the diseased plants does not disclose the presence of any recognizable parasite.

Variations parallel in every respect to those described above have been observed frequently by the writer in the mosaic disease of beans.

Approximately 75 per cent, or more, of the plants in these fields became diseased. Frequent observations showed that aphids (*Myzus persicae* Sulz.) were abundant on the lettuce during the time the disease was developing. Similar conditions were observed in April, 1919, when the writer found what appeared to be the same disease in destructive amounts in several fields of head lettuce at Beaufort, S. C., which were at that time nearly ready for harvest. Several growers stated that aphids had been abundant in these fields a few weeks earlier. A disease that seemed identical has also been observed every season for several years in numerous localities in New York State, usually, however, affecting only occasional plants and causing only minor losses. During four seasons (1914–1917) it occurred in practically all fields of lettuce in the vicinity of Rochester, N. Y., where aphids and other insects were usually more or less abundant, while on the same farms lettuce grown during the winter in the greenhouses, where aphids and other insects were held at a minimum by fumigation, was usually entirely free from the disease.

In order to follow up experimentally these observations, which suggested a relation between the mosaic disease and aphids, several insect cages were constructed of cheesecloth, which were large enough to permit the growing of several lettuce plants under each. Lettuce of both the Big Boston and Paris White Cos varieties was grown from seed under the cages in the field at Sanford, Fla., during the winter season of 1920, particular care being exercised to prevent any aphids from reaching the plants except when intentionally placed on them. *Myzus persicae* Sulz. was used in all the experiments.

On February 10 two aphids collected from several mosaic lettuce plants were placed on each of 25 small healthy lettuce plants under an insect cage. When these were examined, on March 8, there were 7 mosaic and 5 healthy Paris White Cos plants and 5 mosaic and 8 healthy Big Boston plants. Twenty-five plants grown under an adjacent control cage, under conditions comparable in every respect except that no aphids had been placed on them, were all healthy, with the exception of one mosaic plant. The plants were still small, having made slow growth on account of cool weather. There were no aphids in the control cage. In the aphid cage there were at least a few aphids on each plant, but they were apparently not numerous enough to interfere materially with normal growth.

Six aphids from a colony on mosaic lettuce plants under a cage were transferred on March 15 to each of 16 healthy, rapidly growing lettuce plants under an insect cage. On March 27 several of these plants showed the first symptom of the mosaic disease, as previously described, and there were several aphids on each plant. On this date all aphids were destroyed by drenching the plants with "Black Leaf 40" solution. On March 31 there were 4 mosaic and 4 healthy Paris White Cos plants and 3 mosaic and 5 healthy Big Boston plants. Sixteen comparable control plants under an adjacent cage were all healthy. On April 15 there were 6 mosaic and 2 healthy Paris White Cos plants and 5 mosaic and 3 healthy Big Boston plants. All the 16 control plants were still healthy. Both cages were free from aphids.

On March 22 three sets of comparable healthy, rapidly growing lettuce plants under three insect cages were treated as follows: Ten aphids obtained from the same colony on mosaic lettuce from which the aphids in the preceding experiment were secured were placed on each plant in cage No. 1. Ten aphids that had presumably never fed on lettuce were collected from a potato field and placed on each plant in cage No. 2. Cage No. 3 was left without aphids, as a control. The first symptom of the mosaic disease was evident on 2 plants in cage No. 1 on March 30 (Pl. 87, B). On April 14 all 4 Big Boston plants and 3 of the 5 Paris White Cos plants in cage No. 1 showed the mosaic disease, while the 9 comparable plants in each of cages No. 2 and 3 were apparently healthy. Aphids were abundant in cages No. 1 and 2 and were lacking in cage No. 3.

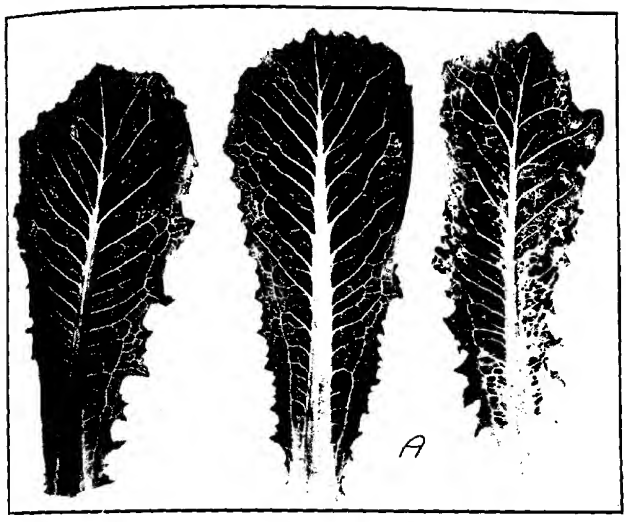
CONCLUSION

There occurs at Sanford, Fla., a serious infectious disease of lettuce, apparently caused by a parasite not capable of isolation through ordinary microbiological or bacteriological technic. The disease has been transmitted experimentally from diseased plants to healthy plants by means of aphids, particularly the species *Myzus persicae* Sulz. From the symptoms and general character of the disease, it should undoubtedly be recognized as a true mosaic disease of lettuce.

PLATE 87

A.—Leaves of Romaine lettuce. Leaf in center from healthy plant; two others from mosaic plants, one showing pronounced type of mottling and the other general yellowish discoloration with few green blotches along larger veins.

B.—Young expanding leaves of head lettuce from experiment started March 22. Leaf on left from healthy plant; two others from plant in early stage of the mosaic disease, showing yellowish discoloration along smaller leaf veins.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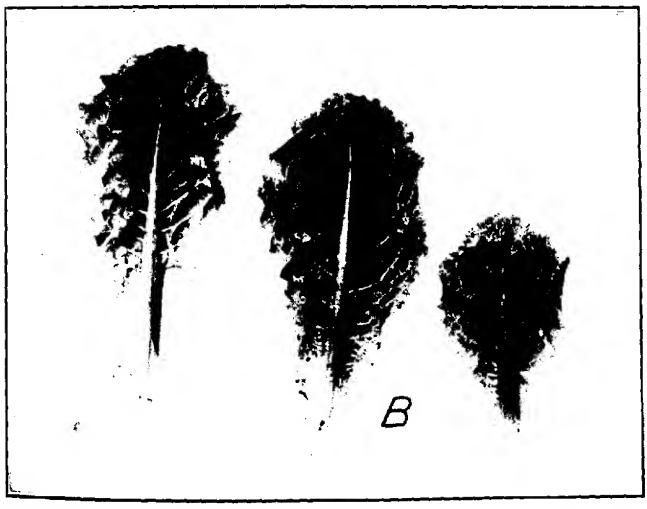
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LECONTE'S SAWFLY,[1] AN ENEMY OF YOUNG PINES

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INTRODUCTION

The following paper on Leconte's sawfly, *Neodiprion lecontei* (Fitch),[1] consists of a detailed description of the various phases of this insect and summarizes the notes on the life and seasonal history. A few notes on the economic importance and the means of control are added.[2]

In describing the larva special care has been taken, and such new terms as have been introduced are carefully explained and illustrated. It is believed that by the introduction of these terms it has been possible to give a more nearly accurate description of the larva and that this terminology will aid in the preparation of descriptions of larvæ belonging to allied groups. The terminology here used is the same as that applied to *Pteronidea ribesii* (Scopoli), *Neodiprion lecontei*, and other sawfly larvæ in a paper ready for publication, and for the reasons therein contained and to avoid possible confusion it seems advisable to continue the use of the same letters to designate the same body areas.

Because of the feeding habits of the larva, Leconte's sawfly is an important enemy to young pine trees in the eastern part of the United States. It is especially injurious to nursery stock. While this paper deals briefly with all of the phases of the insect, more detailed accounts of its life and seasonal history, the damage done, and the means of control have been reserved for future publications of a less technical nature.

DESCRIPTIONS

ADULTS

FEMALE (PL. 88, A)

Length of female 6 to 9.5 mm. Labrum narrowly rounded apically, the surface shining and slightly concave; clypeus broadly subangulately emarginate, apical margin broadly depressed, the basal part convex, with small, poorly-defined punctures; supraclypeal area flattened; antennal foveæ large, shallow, connected with the deep supraclypeal foveæ; lateral foveæ large, circular, deep; middle foveæ and ocellar basin shallow, poorly defined; postocellar area usually well defined, convex, wider

[1] Order Hymenoptera, suborder Chalastogastra, family Tenthredinidae, subfamily Diprioninae.

[2] All the rearing and experimental work on which this paper is based was carried on in the insectaries and nurseries of the Eastern Field Station of Forest Insect Investigations, Bureau of Entomology, located at East Falls Church, Va. The work has been done under the direction of Mr. S. A. Rohwer, specialist in Forest Hymenoptera, and the author is indebted to him for the descriptions of the adults, helpful suggestions, and many of the observations here recorded. Plate 88 was drawn by Miss Mary Carmody, Plate 92 was photographed by H. B. Kirk, and Plates 89 to 91 were drawn by the author.

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posteriorly, somewhat impressed medianly, about two and one-half times as wide as the cephalo-caudad length; postocellar line distinctly shorter than the ocellocular line; antennæ robust, normally 19-jointed but varying from 18- to 21-jointed, apical joints a little more than twice as wide as long, joints 3 and 4 subequal, the basal rami more slender than the apical ones, pedicellum much wider than long; head dulled, with scattered shallow punctures; mesonotum shining, with separate distinct punctures, anteriorly the punctures closer; scutellum with somewhat larger punctures; mesepisternum punctato-reticulate; first parapteron depressed anteriorly and ventrally omitting the depressed area the outline forming an equilateral triangle; tergites, except the ventral aspect, polished, impunctate; last sternite broadly, arcuately emarginate; pad-like apical ventral portion of the sheath a little over four times as long as wide and fitting close to the median ridge of sheath; venation normal. Head, prothorax, and mesothorax rufo-ferruginous; mesosternum blackish to ferruginous; greater part of the mesepisternum sometimes pale ferruginous; metathorax and abdomen black, ventral aspect of tergites whitish, nates and sheath rufo-ferruginous, venter black or in part ferruginous. Legs ferruginous, part of femora and bases of coxæ blackish; bases of tibiæ and basitarsi whitish; occasionally the tibiæ are all whitish. Wings vitreous, subhyaline; venation dark brown. Antennæ black.

MALE (PL. 88, B)

Length 5 to 6.5 mm. Labrum polished, the apical margin rather broadly rounded, clypeus with the apical margin very gently arcuately emarginate, not depressed, the surface sparsely punctured; lateral foveæ practically wanting, other foveæ as in female; ocellar basin represented by a glabrous impression; postocellar area well-defined, subconvex, not impressed, postocellar furrow arcuate; postocellar line slightly shorter than ocellocular line; head with large punctures, those on the front closer, those on the vertex and occiput more widely separated; antennæ 19-jointed; mesonotum with small separate punctures, those of the scutellum rather larger; mesepimeron punctato-reticulate; hypopygidium broadly rounded apically, exceeding the genitalia. Black; labrum pallid; apices of mandibles piceous; legs below trochanters and middle of venter reddish yellow. Wings hyaline, iridescent; venation pale brown.

EGG

Egg 0.25 mm. long by 0.5 mm. broad; envelope very thin, whitish, smooth, shining, translucent, and oval in outline.

LARVA (SIXTH INSTAR) [1]

The following description is prepared from apparently full-grown larvæ from alcohol, approximating 21 mm. in length (Pl. 89, A).

[1] In the description of sawfly larvæ, both structurally and for color, it is necessary that particular areas and regions of a segment or body wall be designated and that the designations adopted be applicable to both the thorax and abdomen of the larva in all its stages. Further, the method, or system, should permit *by addition, elimination, change in shape, armature, and spotting of folds, areas, or regions*, the comparison with other larvæ, and at the same time should avoid possible confusion of meaning. The following is a suggestion for such a terminology and is the one used in the succeeding pages.

An intermediate (second to eighth, inclusive) abdominal segment of *Neodiprion lecontei* (Pl. 91, B, E) consists of tergum, pleurum, and sternum and begins with the transverse tergal fold immediately preceding that above the spiracle.

The tergum is composed of six transverse folds which are considered as representing four primary divisions (A, B, C, D), with one, the third, twice subdivided ($C^{1, 2}$).

The pleurum is divided into three folds—the dorsal anterior one here called the preepipleurite, the posterior one called the postepipleurite, and a ventral one called the hypopleurite—and two areas, one containing the spiracle and the other, armed with a few spines, posterior to and adjoining that containing the spiracle. The area containing the spiracle is at the lower extremity of fold B immediately above the preepipleurite

HEAD (PL. 90, A–E)

STRUCTURAL CHARACTERS.—The dimensions of the head are 2.33 mm. in height (dorsad-ventrad) by 1.75 mm. broad. The capsule (Pl. 90, B, C) is of thin chitin with two openings, the occipital foramen in the posterior wall where the head joins the thorax and the buccal foramen in the venter where the pharynx, mandibles, etc., are situated. The head consists of the following sclerites, areas, and organs: Epicranium, eyes, antennæ, frons, adfrons, pleurostoma, hypostoma, clypeus, labrum,

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and is termed the spiracular area, while the second area, that posterior to the above and armed with few spines, is below folds $C^{1,2,3}$ and is termed the postspiracular area.

The sternum consists of two transverse folds before the hypopleurites, one between and one behind them. The hypopleurites bear the uropods.

These segmental divisions are all rather well defined externally by infoldings of the skin or body wall (Pl. 89, B; 91, D, E), which serve to bear the attachments of certain muscles. These muscles are of considerable value in defining the folds but are not discussed here in detail, since they would require much comparison of forms, bring matter irrelevant to the subject at hand into the paper, and can better be treated fully in a separate paper after further study. It should be said, however, that the studies made thus far seem to bear out the foregoing conclusions and to offer an excellent method by which to limit segments and segment subdivisions and check up homology of the areas, abdomen to thorax, species to species, and larva to adult.

The interpretation of the segmental composition and terminology outlined above is applied to the thorax (Pl. 91, A, D) in the following way: Each of the three thoracic segments (prothorax, mesothorax, and metathorax) is 4-annulate tergally, and the annulations when viewed with reference to ornamentation, shape, position, and relation with one another homologize in order with the primary divisions (A, B, C, and D) of the abdomen, the third, C, not being subdivided.

The pleurum is distinctly divided into four lobes, preepipleurite, postepipleurite, prehypopleurite, and posthypopleurite, in all three segments; and the postspiracular area is present, in approximately its relative abdominal position, in the mesothoracic and metathoracic segments, despite the absence or displacement of the spiracle.

The sternum consists of three small, rather indistinct folds anterior to the leg's basal attachment to prehypopleurite and posthypopleurite.

Further, the transverse circumference of the larva is divided into longitudinal areas of about equal width, (Pl. 91, F).

TERGUM OR DORSUM

The tergum or dorsum in the present paper is intended to designate that portion of the larva which is dorsad of the spiracular and postspiracular areas and which is divided into transverse folds or annulets A, B, C, and D in the thorax, and A, B, $C^{1,2,3}$ and D in the abdomen.

I^a.—Middorsal, a single longitudinal midtergal line.

I.—Dorsal, a pair of longitudinal tergal regions, one to either side of the middorsal line.

II.—Subdorsal, a pair of longitudinal regions, one to each side of the dorsal regions.

III.—Laterodorsal, longitudinal regions, laterad of subdorsal regions.

IV.—Supraspiracular, longitudinal regions, laterad of latero-dorsal regions.

PLEURUM OR LATUS

The pleurum or latus designates that portion of the larva between tergum and sternum.

V.—Spiracular, longitudinal regions, one to each side of the larva and ventrad of the supraspiracular regions, with the abdominal spiracle situated therein in most sawfly larvæ, including *Neodiprion lecontei*.

VI.—Epipleural, longitudinal regions ventrad of spiracular.

VII.—Pleural, longitudinal regions ventrad of epipleural.

VIII.—Hypopleural or lateroventral, paired longitudinal regions, in which are situated the hypopleurites, one to either side of the sternum and ventrad of the pleural regions.

STERNUM OR VENTER

The sternum or venter designates that portion of the larva beneath the body between the uropods. The ventrad projection of the uropods places them with reference to the position they occupy in relation to other structures in the adventral longitudinal areas.

IX.—Adventral, paired longitudinal regions containing the uropods, one protruding from each hypopleurite.

X.—Ventral, a pair of longitudinal sternal regions.

X^a.—Midventral, a single, midsternal, longitudinal line.

epipharynx, tentorium (arms and bridge), hypopharynx, maxillæ, labium, and mandibles.

The epicranium is the largest area of the head, extending from the dorsal margins of the frons and the lateral margins of the adfrons on the anterior wall of the head to the dorsal margin of the occipital foramen and the lateral margins of the hypostoma on the posterior wall. The epicranium is divided dorsally by a rather faint, median line, the epicranial suture (Pl. 90, G), from the dorsal angle of the frons to the occipital foramen, and has a pair of slight, parallel seams beginning near the lateral extremities of the occipital foramen and extending a short distance dorsally. It is moderately spined generally but has concentrations of spines in the areas about the antennæ, eyes, and pleurostomata. The eyes (Pl. 90, A, D) are a single simple pair, one occurring near each of the lateral extremities of the head and slightly below a line drawn through the dorsad-laterad angles of the frons. The antennæ (Pl. 90, F) are paired and occur one each about midway between each eye and the nearest portion of the pleurostoma. They consist of an elongate projecting cone anteriorly and two flat, floating pieces beyond, one of which is usually faintly connected with a narrow band running forward around the cone. The frons (Pl. 90, G) is an inverted, somewhat shield-shaped area and has for its dorsal margin an angle projecting into the epicranium with its apex at about the height of the head's greatest width. Its lateral margins are nearly parallel and about equal in length to the distance of their separation, while the ventral margin is moderately concave. This sclerite is spined according to a rather regular pattern, but the number of spines and their position vary somewhat. The adfrons (Pl. 90, G) consists of an elongate area of thick chitin situated laterad of the frons and separating it from the epicranium. In outline each adfrons is somewhat triangular and supports the dorsal attachment of a tentorial arm and the dorsal or anterior condyle for the mandible. The pleurostomata (Pl. 90, B) are the thickened lateral margins of the epicranium which extend in an arc around the base of each mandible and support at their anterior and posterior extremities the points of articulation of each mandible. The hypostoma (Pl. 90, B) is a centrally narrowing bridge with its dorsal margin formed by the somewhat angular lower rim of the occipital foramen, its ventral margin formed by the slightly curved posterior rim of the buccal foramen, and its lateral limits defined by the slightly curved and thickened ridges running from the lateral extremities of the occipital foramen to the ventral or posterior fossæ for the mandibles. The clypeus (Pl. 90, B) is a dorsally chitinous, ventrally membranous area immediately below the frons and connecting it with the labrum. It is armed with two pairs of spines arranged to form a transverse row. These pairs are separated from each other about two and a half times the distance between the individuals constituting the pair. The labrum (Pl. 90, I) is slightly bilobed

or rounded laterally and subapically but has a median apical concavity and is ornamented with a transverse row of two pairs of spines. These two pairs are slightly farther apart than are the two spines composing each pair. The epipharynx (Pl. 90, E, I) is a thin skin, armed to each side apically, or under each lobe of the labrum, with a series of inwardly diminishing, opposed setæ or blades, lacking symmetry, which often vary somewhat in number and arrangement. The tentorial arms (Pl. 90, B, C) are a pair of supports or struts diverging to the widely separated pair of adfrontal triangles from the tentorial bridge (Pl. 90, B, C), which is a thickened central attachment of the hypostoma. The hypopharynx (Pl. 90, E, J), or floor of the mouth, rests between and beyond the paired maxillary laciniæ and is a thin membrane, minutely ornamented. Each maxilla (Pl. 90, J–N) is composed of cardo, stipes, palpifer, 4-jointed palpus, galea, and lacinia. The labium (Pl. 90, J, K, O) is composed of submentum (or mentum and submentum fused), mentum (or labial stipes), ligula, and, to each side of the latter and attached basally to the mentum (or labial stipes), a palpiger surmounted by 2-jointed palpus. The mandibles (Pl. 90, H) are 5-toothed.

COLOR.—The head capsule is orange-brown, excepting the spots surrounding the eyes, which are black, and a part of the clypeus, which is dark brown. The labrum is pale brown with its entire margin darkened, the chitin of the maxillæ and labium is brown to blackish, while the epipharynx, hypopharynx, and ligula are pale white with their armatures pale brown.

THORAX

STRUCTURAL CHARACTERS.—The prothorax (Pl. 91, A) when examined exteriorly and in its normal position appears to consist dorsally of but two or three annulets, C and D always and B sometimes. This is due to the constriction of the anterior circumference of the segment in its connection with the head. An examination of the skin infoldings (Pl. 91, D), however, will reveal all four of the primary divisions. On the posterior margin of the segment, but caudad-ventrad of B, which is always distinct supraspiracularly, there is a large, rather elongate area in which the large thoracic spiracle is situated. Ventrad of B and anterior to this spiracular area is the preepipleurite; below the preepipleurite and the spiracular area is the postepipleurite; and under the latter comes the posthypopleurite, anterior to which, and rather strongly chitinized, is the prehypopleurite. The prehypopleurite and posthypopleurite support the 4-jointed legs. That part of the venter not occupied by the prehypopleurite and posthypopleurite is divided by three transverse folds into four annulations, the first annulation with a pair of latero-ventral, chitinized areas, extending one from the base of each leg forward to the occipital foramen, called neck plates. B supraspiracularly, C, preepipleurite, postepipleurite, prehypopleurite, posthypopleurite, the leg joints, and the second and third sternal folds are armed with spines.

The mesothorax (Pl. 91, A, D) is not constricted in circumference anteriorly and is readily seen to be composed of the four primary tergal annulets, a small fold ventrad of A and anterior to the preepipleurite, the postspiracular area, preepipleurite and postepipleurite, prehypopleurite and posthypopleurite, 4-jointed legs, and four transverse sternal folds. A, B, C, preepipleurite, postepipleurite, prehypopleurite, posthypopleurite, leg joints, and third and fourth sternal folds are armed with spines.

The metathorax (Pl. 91, A, D) is similar to the mesothorax, except that the small fold anterior to the preepipleurite and ventrad of A is larger and bears hidden on its posterior surface an exceptionally small spiracle.

COLOR.—The prothorax is whitish with the following exceptions: A supraspiracular black spot on B; black prehypopleurite and leg joints; and a pair of black sternal neck plates.

The mesothorax is whitish with the following exceptions: A subdorsal black spot on A, B, and C; a spiracular and supraspiracular black spot on B and C and the postspiracular area; a black preepipleural spot; and black prehypopleurite and leg joints.

The metathorax is similar to the mesothorax.

ABDOMEN

STRUCTURAL CHARACTERS.—In an intermediate (second to eighth, inclusive) abdominal segment (Pl. 89, B; Pl. 91, B, E) the tergum consists of six transverse folds (A, B, $C^{1, 2, 3}$ and D). The pleurum is divided into preepipleurite, postepipleurite, hypopleurite, spiracular area ventrad of B and bearing the spiracle, and postspiracular area posterior to the spiracular area and below $C^{1, 2, 3}$. The sternum is composed of two transverse folds before the hypopleurite and one behind it. The uropods project from the hypopleurites. Annulets A, B, and C^2, postspiracular area, preepipleurite, and postepipleurite are armed with spines.

The first and ninth abdominal segments are similar (Pl. 91, C) but lack a well-developed hypopleurite and uropod on venter and have four transverse sternal folds.

The tenth abdominal, or anal segment (Pl. 91, C) consists tergally of a large undivided area termed the epiproct, or anal plate; pleurally, of a somewhat triangular fold situated in the anterior portion of the segment similar to the preepipleurite (the anal opening occurring transversely across the apex of the segment); and sternally, of the postpedes, the area from which they spring, and the postcallus below the anus. All folds and areas, except the postpedes, are armed with spines. The area around the base of the postpedes is, however, but slightly spined or haired.

COLOR.—The intermediate (or second to eighth, inclusive) abdominal segments are whitish, with the following exceptions: A subdorsal black spot occurring and diminishing posteriorly on A, B, C^1, and C^2; a supra-

spiracular black spot on B, C^1, C^2, and the dorsad extremity of post-spiracular area; a black spot on preepipleurite; and sometimes a small blackish spot on postepipleurite. The first and ninth abdominal segments are similar but have the preepipleural spot smaller and the post-epipleural spot almost always absent. The tenth abdominal, or anal, segment is white but with the epiproct black.

LARVAL INSTARS

The larval life of sawflies of the group to which this species belongs is divided into two distinct periods by a change of objective. The form and color of the larvæ differ considerably in these two periods. In the first period the larvæ are active and, as they devote most of their energy to feeding, change rapidly in size. There are usually six molts. In the second period the larva is more contracted, less active, and devotes its energies to seeking a place for and constructing the cocoon. No feeding is done in this second period and there is no molting. This second period is generally termed the prepupal period, but other American writers have referred to it as the ultimate stage.

These periods, stages, or instars are measured by the hatching of the larvæ from the egg and by the subsequent sheddings or moltings. The larva molts after slightly varying passages of time, the extent of which will be discussed later; and the molting, as a rule, is accomplished by the longitudinal splitting of the prothoracic and mesothoracic skin mid-dorsally, the breaking of the head capsule along the epicranial suture, and the separating of the frons from the epicranium and the adfrontal triangles. Through the opening thus formed the larva in its new skin endeavors to extract itself from the old, and if successful begins feeding anew, leaving the exuvia attached by the anal end to the needle.

The following descriptions of stages and approximate length of each are the summary of notes from numerous rearings of larvæ in quantities, since it has been found that isolation of larvæ not only tends to retard development but often causes death. This method makes impracticable an absolutely accurate account of the time spent by particular larvæ in each stage. The first appearance of shed skins and of what seemed to be a new stage was, however, recorded and was utilized for description and as an index for these approximations.

The larvæ hatch from the eggs with slightly varying periods of incubation and develop at such different rates that following the first molt there are always two and more often three or more stages present at one time. From about the fifth stage a difference in size of the larva, dependent upon sex, becomes noticeable, to confuse further an endeavor to determine stages accurately.

All the stages are similar to the sixth stage, except as noted in the following descriptions. A detailed description of the sixth instar has already been given under the heading "Larva."

FIRST INSTAR

STRUCTURAL CHARACTERS.—The larva increases in length from about 2 mm. at hatching to about 5 mm. at the beginning of the second stage. In proportion the thorax is slightly large for the abdomen, whereas the head is large for the thorax. The body spines are obsolete, and the spiracles are unusually large, having the appearance of being expanded.

COLOR.—The head is brownish with the eye spots, the labial and maxillary chitin, and the apices of the mandibles blackish. The body is unspotted and previous to feeding is entirely yellowish gray, but upon the filling of the alimentary canal it appears green or lead green. The thoracic leg joints are blackish.

SECOND INSTAR

STRUCTURAL CHARACTERS.—The second stage develops in length from about 5 mm. to 7.5 mm. The head is still large but the thorax and abdomen are nearly normal in their relation to each other. The spiracles are now about normal in their appearance, and the spines are becoming fairly distinct.

COLOR.—The head is brownish with the eye spots and the labial and maxillary chitin blackish. The body is unspotted and pale yellow-green, with the spines appearing faintly grayish, and the thoracic legs are blackish.

THIRD INSTAR

STRUCTURAL CHARACTERS.—The larva in the third instar grows in length from 7.5 mm. to 10 mm. The head is still large, wider and higher than the thorax, and the body spines are now prominent.

COLOR.—The head is brownish to brownish black, with the eye spots black but not conspicuous. The labrum, apices of mandibles, and chitin of the labium and maxillæ are brownish black. The thorax and abdomen are pale, usually unspotted, but in some larvæ with very faint gray supraspiracular spots and epiproct. The thoracic leg joints are black.

FOURTH INSTAR

STRUCTURAL CHARACTERS.—The larvæ of the fourth stage lengthen from 10 mm. to 12.5 mm. The head is now about normal size in relation to the body.

COLOR.—Approximately the same as in the sixth stage. The head varies from brownish to orange and the body is normally spotted with gray black.

FIFTH INSTAR

STRUCTURAL CHARACTERS.—The fifth stage increases in length from 14 mm. to 18 mm. Structure as in sixth stage.

COLOR.—Same as the sixth stage except that the head sometimes has more brown and the body markings appear in some instances proportionally larger and a deeper black than in the sixth stage.

SIXTH INSTAR

In this instar the larva grows from 18 mm. to about 22 mm. For characters, see previous detailed descriptions of larva.

PREPUPA

The prepupa, or seventh larval instar, is the nonfeeding, cocoon-spinning stage in which the larvæ search out a suitable place to spend their quiescent period. In size they usually measure from 10.5 mm. for one which has spun a male or small type of cocoon, to 12 mm. for one which has spun an average size large type or female cocoon.

HEAD

STRUCTURAL CHARACTERS.—The head is 2 mm. in height (dorsad-ventrad) by 1.6 mm. broad, and except for being somewhat smaller is similar to that in the preceding, or sixth, stage.

COLOR.—The head is pale whitish, usually grayish across the dorsum above the eyes. The eyes are pale and are placed somewhat dorsad-caudad of the center of the black oval spot surrounding them. The antennal joints are inconspicuous, being yellowish white on a white membrane. The frons, adfrons, clypeus, labrum, labium, and maxillæ are all pale, the heaviest chitin appearing only yellowish white while the mandibles are pale excepting the teeth, which are brownish black.

THORAX

STRUCTURAL CHARACTERS.—The thorax is similar to that of the sixth stage larva.

COLOR.—The thorax is about the same as that of the sixth stage except that the skin is pale white rather than yellowish white, with spots grayish black rather than black; the mesothoracic and metathoracic subdorsal spots are absent on fold C and very faint on B; and the legs are entirely white.

ABDOMEN

STRUCTURAL CHARACTERS.—The abdomen is similar to that of the sixth stage.

COCOON

The cocoon is a tough, single-walled, papery, red-brown cylinder with hemispherical ends. The exterior, which is darker and less glossy than the interior, shows some coarse threads and often has particles of sand or other surroundings adhering to it. The cocoons vary in size for both sex and individuals. In a number examined, the female, or larger cocoons, varied from 9.5 to 11 mm. in length and from 4.5 to 5 mm. in diameter, averaging 10.3 mm. long by 4.6 mm. in diameter. The male, or smaller cocoons, vary from 7 to 7.8 mm. in length and from 3.2 to 3.5 mm. in diameter, averaging 7.5 mm. long by 3.4 mm. in diameter.

PUPA

Little is known of the pupa stage, but without doubt it is of short duration, since pupæ are rarely found when cocoons are cut open, either shortly after being spun or up to the time they are a year old and have practically all produced adults.

The following descriptions are prepared from a female pupa.

STRUCTURAL CHARACTERS.—The pupa is similar to, though somewhat larger and less hardened than the unemerged adults. The flagellum of the antenna varies from 19 to 20 in the number of joints in the specimens counted. The appendages are folded in or toward the venter with the second pair of wings under the first pair which extend caudadventrad. The shed prepupal skin is attached loosely to the apex of the pupa's abdomen.

COLOR.—The pupa is entirely yellowish, the eyes, apices of the mandibles, and antennæ being the first parts to darken with the development of the adult.

UNEMERGED ADULT

The approach of the pupa toward the mature adult is accompanied by a darkening, or coloration, and hardening of the body wall, which before issuance becomes almost complete, and by the shedding or removal of the pupal membrane or skin, by a reduction in size, and by an increase in activity.

The following descriptions are prepared from an unemerged female adult.

STRUCTURAL CHARACTERS.—The unemerged adult is similar to the mature adult, and the shed pupal skin is attached loosely to the apex of the abdomen.

COLOR.—The head is yellowish brown, with the eyes leaden, the antennæ brownish, the apices of the mandibles brown, and the labium and maxillæ yellowish white. The greater part of the thorax is yellow to yellowish white, but some of the posterior sclerites (mesothorax in part and all of the metathorax) are brownish. The wings are nearly completely developed with their veins brownish, and the legs, excepting small portions, are yellowish white. The abdomen has the tergites (except intersegmental skin) blackish with a broad, white, longitudinal band along the spiracles; the pleural line white; the sternites white medially, brownish near pleural line; and the reproductive parts mostly yellowish.

LIFE AND SEASONAL HISTORY

The length of life of a colony, or the time between the depositing of the first egg and emergence of the last adult, may be approximately either 12 or 14 months—12 months when the eggs are laid in the late summer or early fall and 14 months when the eggs are laid in the later spring or early summer. The length of life of a single colony has been given the name "colony period."

From the cocoons of a single colony there are two periods of adult emergence. The first period is termed "brood A," and the second "brood B." When the colony period begins in late spring or early summer, brood A emerges in the late summer and early fall of the same year and brood B emerges in the late summer and early fall of the following year, making the length of the colony period 14 months. When the colony period begins in the late summer, brood A emerges in the spring and early summer of the following year and brood B emerges in the late summer and early fall of the same year as brood A, making the length of the colony period 12 months.

Thus (see year II in fig. 1) we may have adults of brood B of the first colony period, brood B of the second colony period, and brood A of the third colony period existing in the late summer of the same year. In

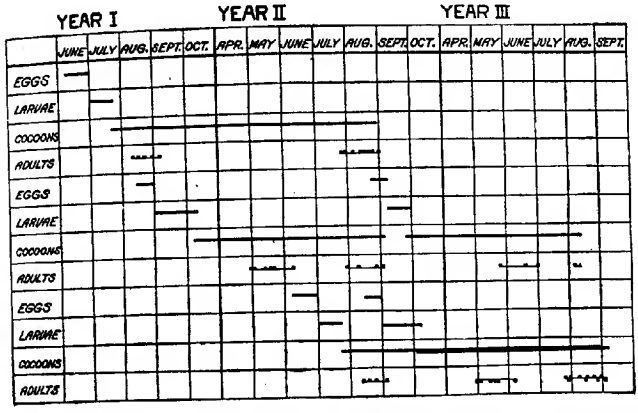


FIG. 1.—Chart showing life and seasonal history of *Neodiprion lecontei* through the active period of three years (November to March omitted, the insect being in the cocoon during this period).

the spring, however, it is possible only to have brood A, but these may be from different colony periods (see year III in fig. 1).

The eggs are laid in a row of slits along one of the serrated edges of the leaf (Pl. 92, B). These slits, the work of the female's saw, are about 1.5 mm. long and 0.8 to 0.9 mm. deep and have an interval between them about equal to their length. They are somewhat shoe-shaped, the opening or slit not entirely covering the pocket, and deepen slightly toward the apex, or toe. These egg punctures are rather conspicuous, appearing yellowish against the green of the undisturbed leaf tissue and becoming brownish with age. Usually the leaves containing eggs die and become noticeable some time after the hatching of the larvæ.

In cage experiments the number of eggs laid by single females varied from 25 to 178, with an average of 82. In six virgin females dissected the number of eggs varied from 58 to 218, with an average of 139, so it is

certain that in these experiments the maximum number of eggs was not obtained. Available data indicate that approximately two-thirds of all the eggs laid produce larvæ.

As a result of there being two periods of adult emergence there are two periods of oviposition and incubation during the year, coincident with those of issuance, the first occurring in the late spring and early summer (particularly May and June) and the second in the late summer and early fall (late July, August, and early September).

The period of incubation as determined by the time elapsing between the laying of the first egg and the hatching of the first larva varies from 13 to 21 days with an average, from six experiments, of 16 days.

For the first 5 or 6 days after oviposition very little change is noted in the eggs, but beginning with the seventh or eighth day a gradual swelling is evident, so that by the ninth day there is a slight separation of the sides of the mouth of the egg pocket. This separation increases until it is 0.5 mm. in breadth shortly before the egg hatches and the larva emerges.

The length of the larval feeding period, from the hatching of the eggs to the appearance of the first prepupa, varies from 25 to 31 days, with an average of 28 days.

During the whole of the feeding period the larvæ are gregarious and show little or no tendency to disperse. If disturbed while feeding they throw back the head and thorax and remain motionless in that attitude, attached to the needle only by the uropods.

The larvæ for the first, second, and third stages eat only the epidermis and the immediately adjoining tissue of the needles. The approximate length of the first stage is 6 days, of the second 5 days, and of the third 5 days. Beginning with the fourth stage and continuing through the sixth, the larvæ eat the whole of the needle and occasionally portions of the tender bark on the young twigs (Pl. 92, A). Field observations on the feeding upon the bark seem to indicate, however, that the species of the tree may have more influence than the amount of foliage available. The bark of the jack pine (*Pinus banksiana*) in Wisconsin and Virginia was usually fed upon, even though there was plenty of foliage available. The approximate length of the fourth stage is 5 days, of the fifth 4 days, and of the sixth 4 days.

Following the larval feeding period comes the prepupal instar, a larval, nonfeeding, cocoon-spinning, quiescent stage. The prepupæ first seek a suitable place and then spin their cocoons. In nature the cocoons have only been found several inches under the surface of the ground under the tree attacked.

After the cocoon is made the insect remains for a comparatively long time as a prepupa, but shortly prior to the time of its emergence it transforms to the pupa and then develops rapidly into the adult stage, which

cuts an end completely, or nearly so, from the cocoon, and issues. It is in the cocoon that this insect passes the winter.

The length of the cocoon period, from its spinning until the issuance of the adult, varies with the character of the colony. If the cocoons are made by the larvæ hatching from eggs laid in early summer (May or June) there will be an emergence, called brood A, in the late summer or early fall of the same year (late July, August, and early September) and a second emergence from cocoons made by larvæ of the same colony, called brood B, in the late summer and early fall of the following year. In such instances the length of the period between the first cocoon and the first adult of brood A varies from 13 to 23 days, averaging 18 days; and the period between the first cocoon and the first adult of brood B varies from 364 to 379 days, averaging 371.

If, however, the cocoons are made by the larvæ hatching from eggs laid in the late summer or early fall the emergence of brood A will not take place until late spring and early summer of the following year, while brood B will emerge in the late summer and early fall of the same year as brood A. In this instance the time elapsing between the making of the first cocoon and the first emergence of brood A varies from 205 to 242 days, averaging 218 days, while that between the making of the first cocoon and the first emergence of brood B varies from 292 to 342 days, averaging 309 days.

The female adults seem to predominate throughout any period of emergence and in a whole colony by the ratio of 3 to 1. Although the females predominate for any given period of emergence or brood in the sense in which it has been used in this paper, it is not unusual to find that at either the beginning or end of the period males will emerge in the majority.

EFFECT OF METEOROLOGICAL CONDITIONS

Eggs laid in late July and early August—that is, during the warmest periods—hatch more quickly than those laid later or earlier in the year. The particular period of the year, however, or the heat has not been proved to be directly responsible for the speed of development, although from temperature readings during the periods of incubation this would seem to be a fact. For example, in June, 1917, when the mean temperature during the incubation period was 71.23° F., with a mean minimum of 60.14°, eggs hatched in 21 days; in mid-August, when the mean temperature during incubation was 74.59°, with a mean minimum of 63.59°, the eggs hatched in 18 days; and in late July and early August, 1917, when the mean temperature of the incubation period was 78.8°, with a mean minimum of 67.69°, eggs hatched in 13 days.

Further, the relation of humidity to development must be considered, and it would seem from our records that high humidity tends to retard incubation. For example, in June, 1917, when the average humidity

during the incubation period was 74.30 per cent, eggs hatched in 21 days; in mid-August, when the average humidity during incubation was 67.80 per cent, eggs hatched in 18 days; and in late July and early August, 1917, when the average humidity of the incubation period was 65.57 per cent, eggs hatched in 13 days.

TABLE I.—*Record of temperature and humidity during incubation period of Neodiprion lecontei*

Date.	Length of incubation period.	Mean maximum temperature.	Mean temperature.	Mean minimum temperature.	Mean relative humidity.
1917.	*Days.*	°F.	°F.	°F.	*Per cent.*
June 9 to 29	21	82.33	71.23	60.14	74.30
July 30 to Aug. 11	13	89.92	78.80	67.69	65.57
Aug. 15 to Sept. 1	18	85.36	74.59	63.83	67.80

It is highly probable that normal development or acceleration is due to the favorable combination or balance of both temperature and humidity and that there are definite limits beyond which heat or moisture would be either insufficient or excessive and result in retardation or death.

Notes on the response of larvæ of this species to meteorological influences are few and somewhat contradictory. The author has observed a decided retardation of activity, feeding, and development, when damp, cold, and cloudy weather occurs in the warm season, and a corresponding acceleration on sunny days. Colonies were found feeding near Falls Church, Va., on November 5, the day being bright but after a heavy frost, while S. A. Rohwer records "nearly full-grown larvæ feeding on the sheltered side of a tree even though it was below freezing and snowing hard," near Trout Lake, Boulder Junction, Wis., on September 21, 1913.

MATING AND COPULATION STUDIES

The females occasionally are, or seem to be, active in finding a mate, but more frequently they appear to resist the attempts to mate offered by the male, sometimes cutting off portions of his antennæ and legs with their mandibles. In those instances where copulation was observed there were no preliminary attentions or courtship. Intercourse takes place with the pair in positions in which their abdomens are opposed. It was observed once that the male arrived in position by crawling over the female from head to posterior end. When his abdomen had reached the end of the female's he swung his under hers. During copulation the wings are held flat against the body; the legs are spread rather far apart, the forelegs projecting anteriorly, the middle legs slightly anteriorly, and the hind legs posteriorly; and the antennæ are usually moved slowly, up and down.

Rohwer[1] gives the following description:

Copulation lasts about 100 seconds and is accomplished by the two individuals facing in opposite directions and the extreme end of the male abdomen being bent at an obtuse angle because of the truncate abdomen of the female. The hypopygidium of the male fits over the knob at the base of the sheath, the harpes grasp the sides of the knob in the manner of a ball and socket joint, while the position occupied by the parapenes, sagittæ, volsellæ, and penis valves, was not observed.

OVIPOSITION STUDIES[2]

After locating a suitable place for ovipositing, the female stands with her legs grasping the needle, her abdomen bent ventrally so that its apex comes in contact with the needle at a point between the mesothoracic and metathoracic tarsi. She seems to start the incision with the lance as well as the lancets by pulling or sliding these away from her along the

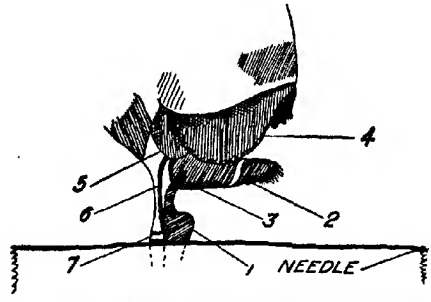


FIG. 2.—Position of end of abdomen of female when ovipositing, showing the various parts and their position: 1, lance; 2, apical part of sheath; 3, basal part of sheath; 4, nates or ninth tergite; 5, eighth sternite; 6, chitinized rods at base of lancet; 7, lancet.

needle in a fashion suggesting an attempt to catch a sharp point or tearing edge in the tissue. After starting the incision she withdraws the lance slightly and appears to use it to guide the lancets and to keep the latter pressed against the front of the cut (fig. 2). After the insertion of the lance and the lancets the female straightens or raises the ventrally bent end of her abdomen, causing the ovipositor to form an abrupt angle with it.

The chitinized basal rods of the lancets run along the chitinized ventral side of the lance and turn into the abdomen towards the ninth tergite. Their up and down motion seems to be controlled by a somewhat side to side movement of the nates, or ninth tergite. The lancets work opposite each other except at withdrawal, when they are worked together up and down and back, following the lance through the arc of the cut they have

[1] ROHWER, S. A. THE MATING HABITS OF SOME SAWFLIES. *In* Proc. Ent. Soc. Wash., v. 17, no. 4, p. 195–198, fig. 1, pl. 22. 1915.
Page 196: Diprion leconteï.

[2] Terminology used here is that adopted in a recent paper (still in proof) by S. A. Rohwer.

just completed. As the ovipositor is removed from the cut the female squats over the freshly made opening and probably at this time deposits the egg. The deposition of the egg could not be seen, but it is believed that the egg does not descend through the ovipositor but that it is dropped in place, leaving the body of the female through the spread bases of the ovipositor, before the ovipositor is completely withdrawn.

The following is an account of the time spent by one female in each of the different steps in the laying of an egg: In scratching the surface of the needle endeavoring to start the incision, she spent 2 minutes and 13 seconds; in working the lance and lancets into the tissue, she spent 22 seconds; in beginning the pocket the female, with her abdomen bent and close to the needle, worked for 27 seconds; and on the remainder of the cutting of the pocket, with her abdomen raised, she worked 1 minute and 49 seconds. The removal of the ovipositor and the deposition of the egg were accomplished in 16 seconds.

PERIODIC APPEARANCE

Leconte's pine sawfly appears and disappears periodically. For several years this species will be very abundant; then for a few years it will become rare. The cause for this periodic disappearance has not been determined, but it seems likely that some factors other than parasitism play an important rôle, because we have no records which give a sufficiently high percentage of parasitism to lead one to believe that this is entirely responsible for a great reduction of the species. Investigation of certain other means of natural control has thrown no light on the subject.

PARTHENOGENESIS

Experiments to determine if this species can reproduce parthenogenetically are inconclusive. In all these experiments only unfertilized females of both emergence periods of brood A were used, and although all of them were failures the information acquired is inadequate to prove that the adults of this brood can not reproduce parthenogenetically. Eight experiments were performed, six of which produced eggs while two failed entirely. In two experiments conducted under especially favorable conditions the eggs hatched but the young larvæ died without molting. It is thus possible to state that females of brood A of this species can and will lay eggs unfertilized and that these unfertilized eggs will hatch, but in no experiments have these larvæ produced adults.

HOSTS

This species appears to have three primary or preferred hosts and a quantity of secondary or possible hosts. The primary hosts as determined by observations in the field and the nursery are: Jack pine (*Pinus banksiana*), which was subject to attack in Vilas and Oneida

Counties in Wisconsin, at Kanawha Station, W. Va., and in the experimental nursery at East Falls Church, Va.; red pine (*P. resinosa*), which was commonly attacked in Vilas and Oneida Counties, Wis., and has been recorded by a correspondent as being attacked at Hyde Park, Dutchess County, N. Y., but which in experiments for oviposition by adults and as food for larvæ conducted in the nursery at East Falls Church, Va., has always led to failures; and scrub pine (*P. virginiana*), which is the native host of this insect through northern Virginia, Maryland, and Pennsylvania.

The secondary or possible hosts can not be ranked as complete hosts capable of supporting the insect through all its various stages or as entirely acceptable to females for oviposition. They have been determined by observation in the field and nursery, from correspondence and literature, and through experimentation. They are white pine (*Pinus strobus*) in Wisconsin and at Reading, Pa.; Scotch pine (*P. sylvestris*) at Reading and Austin, Pa.; loblolly pine (*P. taeda*) Annandale, near Falls Church, Va., and Clinton, La.; shore pine (*P. contorta*) at Kanawha Station, W. Va.; silver pine (*P. monticola*) in the nursery at the Eastern Field Station; mugho pine (*P. mughus*), West Chester, Pa.; *P. eldarica*, Yarrow, Md., chosen in the field and nursery; western yellow pine (*P. ponderosa*), used in experimentation (confining adults in a cage upon the young tree); and longleaf pine (*P. palustris*),[1] Austrian pien (*P. austriaca*),[2] and American larch (*Larix americana*),[3] mentioned in literature and correspondence.

PARASITES

Neodiprion lecontei is subject to attacks by both parasitic insects and a wilt. Four species of hymenopterous and four species of dipterous adults have been reared from the cocoons of this species, but neither egg parasites nor parasites which emerged from uncocooned larvæ have been obtained. The hymenopterous parasites were determined by S. A. Rohwer as *Exenterus diprioni* Rohwer, *Lagorotis diprioni* Rohwer, *L. virginiana* Rohwer, and *Perilampus hyalinus* Say. Of these parasites *L. diprioni* Rohwer is much the most abundant species, and *Perilampus hyalinus* Say is probably a hyperparasite. The dipterous parasites were determined by C. T. Greene as *Phorocera claripennis* Macquart, *Adomonita demylus* Walker, *Neopales maera* Van der Wulp, and *Spathimeitenis spinigera* Townsend.

The wilt of the larvæ was probably a bacterial disease and was found in Wisconsin by S. A. Rohwer, in 1912. The larvæ attacked were readily distinguished by their lack of vigor and their white tracheal system,

[1] Larvæ sent in by a correspondent from Pinehurst, N. C., with the following note: "Eating the pine needle of the longleaf pine in this vicinity."

[2] RILEY, C. V. NINTH ANNUAL REPORT ON THE NOXIOUS, BENEFICIAL, AND OTHER INSECTS OF THE STATE OF MISSOURI, p. 32–33. , Jefferson City, Mo. 1877.

[3] "When forced to, defoliate and girdle," in letter from W. D. Barnard, Boulder Junction, Wis.

which was conspicuous early in the disease when the larvæ were yellow and more noticeable later when the larvæ became darkened. The wilt was rather widespread in this locality of infestation, but though it killed a considerable quantity of the larvæ yet its success was limited.

From our notes and rearing records it would seem that none of the insect parasites were abundant enough nor was the wilt sufficiently distributed and infectious to account for the periodic disappearance of this species. It is certain that neither any nor all of these natural checks are sufficiently numerous or effective to admit disregard of the artificial control measures suggested below.

DISTRIBUTION

Neodiprion lecontei was described by Fitch from specimens collected in New York, while Riley and Norton mentioned specimens coming from Ridgewood, N. J. The localities represented in the United States National Museum collection are Baltimore, Md., and Virginia (near the District of Columbia), material collected by Theo. Pergande; and Long Island, N. Y., material collected and reared by H. G. Dyar. The "Guide to Insects of Connecticut" [1] records the sawfly from Middletown, Hampton, and Stamford, for that State. To these localities, through collecting by members of the Bureau of Entomology and correspondents, the following localities have been added (fig. 3):[2]

CONNECTICUT: Cheshire, Deep River, Ellington, New Haven, Norfolk.
DISTRICT OF COLUMBIA: Throughout.
LOUISIANA: Clinton.
MARYLAND: Yarrow, Plummers Island.
MICHIGAN: Remus.
MISSISSIPPI: Orange Grove.
NEW YORK: Hyde Park (Dutchess County).
NORTH CAROLINA: Pinehurst.
PENNSYLVANIA: Austin, Linglestown, Reading, West Chester.
VIRGINIA: Falls Church and vicinity (generally throughout Arlington and Fairfax Counties).
WEST VIRGINIA: Kanawha Station.
WISCONSIN: Generally throughout Oneida and Vilas Counties.

ECONOMIC IMPORTANCE

This species does considerable damage to both natural reproduction and nursery stock by defoliating the trees. Complete or nearly complete defoliation before late summer usually kills that part defoliated;

[1] VIERECK, Henry Lorenz, et al. GUIDE TO THE INSECTS OF CONNECTICUT. PART III. THE HYMENOPTERA, OR WASP-LIKE INSECTS, OF CONNECTICUT. Conn. State Geol. and Nat. Hist. Survey Bul. 22, p. 44. 1916.

[2] Since this manuscript has been prepared this species has been received from the following additional localities:
CONNECTICUT: Hartford.
FLORIDA: Orlando.
NEW HAMPSHIRE: Wonalancet.
PENNSYLVANIA: Clearfield, New Germantown.

and since this insect shows a very decided preference for young trees, and the larvæ often are numerous enough to strip the tree entirely of leaves, many young pines are killed by this work alone. Trees not completely denuded often die because in their weakened condition they are attacked by secondary insect enemies. When there is incomplete defoliation and the tree recovers it is often stunted or misshapen and is of little commercial or ornamental value.

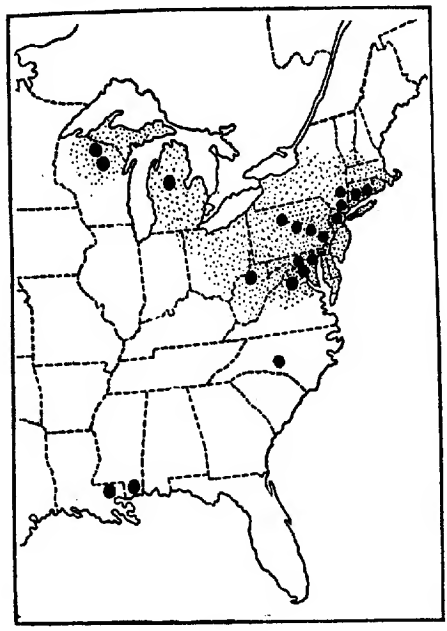


FIG. 3.—Distribution of *Neodiprion lecontei*. The larger dots indicate places from which specimens have actually been received. See also footnote 2, p. 758.

MEANS OF CONTROL

The control of this species depends largely on the extent and location of the infestation. In large areas of either natural or artificial reproduction, control because of its expense can not be generally practiced, but rangers and lumbermen should make it a practice to destroy the colonies of these larvæ whenever they are found. The easiest way is to knock the larvæ from the trees and crush them with the foot.

In nurseries and in parks the control, in case of heavy infestation, can best be attained before the larvæ are full-grown and should consist of thorough spraying. An arsenate of lead spray of 2 pounds of powder to 50 gallons of water (or a ratio of 1 to 12) should be satisfactory. On larvæ which are discovered when young, less than ⅜-inch long, nicotine

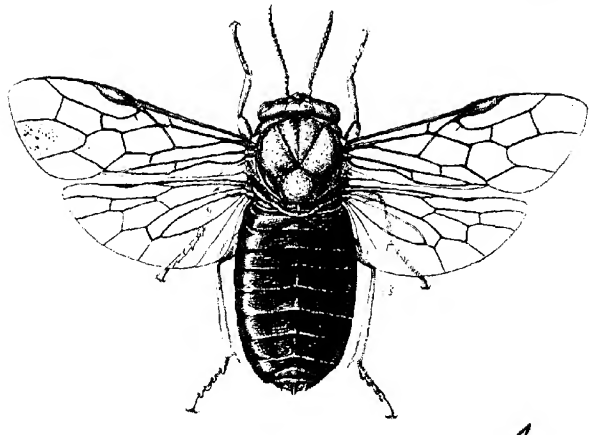
sulphate is a fairly satisfactory spray to use; however, because of the resistance of conifers to arsenical sprays and because an arsenical treatment gives more certain results, it is probable that the spray first recommended should be used almost exclusively. In scattered infestations hand picking or knocking the larvæ from the trees and crushing them will be found to be much more economical and at least as effective.

Whenever these insects are observed in any locality and control measures are practiced against them, it is important that the territory be carefully surveyed for the following 14 months, since it is possible that some larvæ may have escaped the treatment and have spun cocoons. This possibility makes watchfulness necessary over the entire colony period of the species in order that an emergence of adults from these cocoons may not reestablish the infestation.

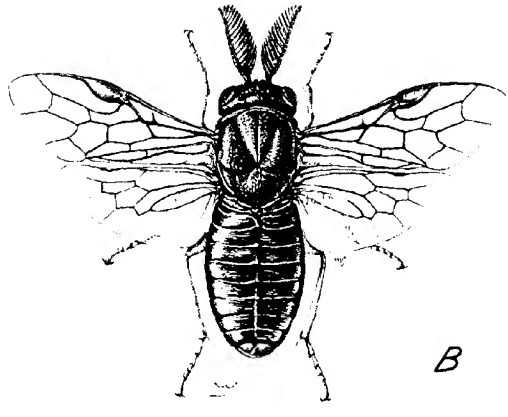
PLATE 88

Neodiprion lecontei:

A.—Adult female.
B.—Adult male.



A



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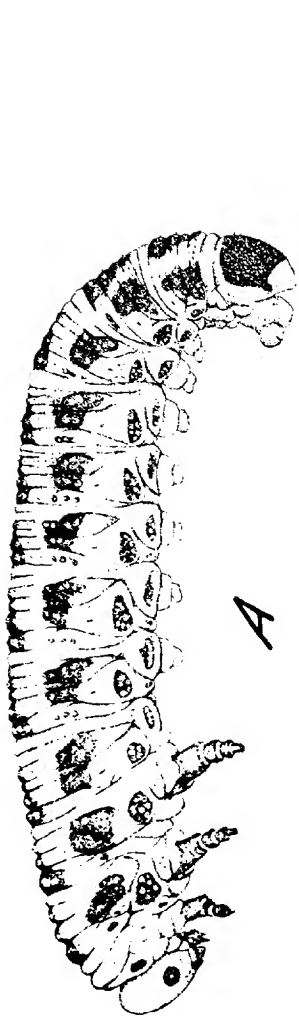
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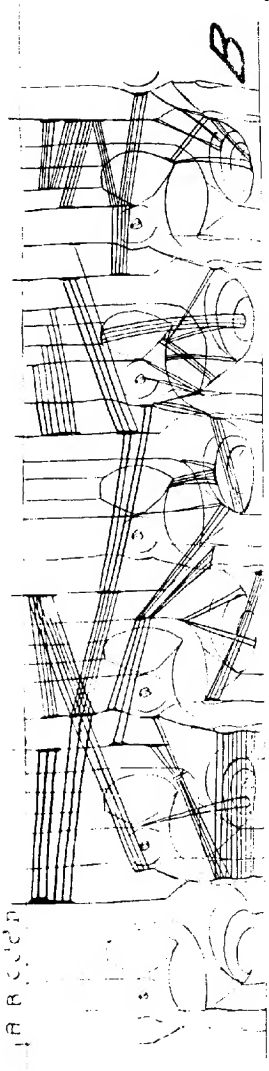

B

PLATE 89

Neodiprion lecontei:

A.—Larva.

B.—Sixth-stage larva: The muscles of a single abdominal segment distributed over several segments to show their numbers, position, and attachment.

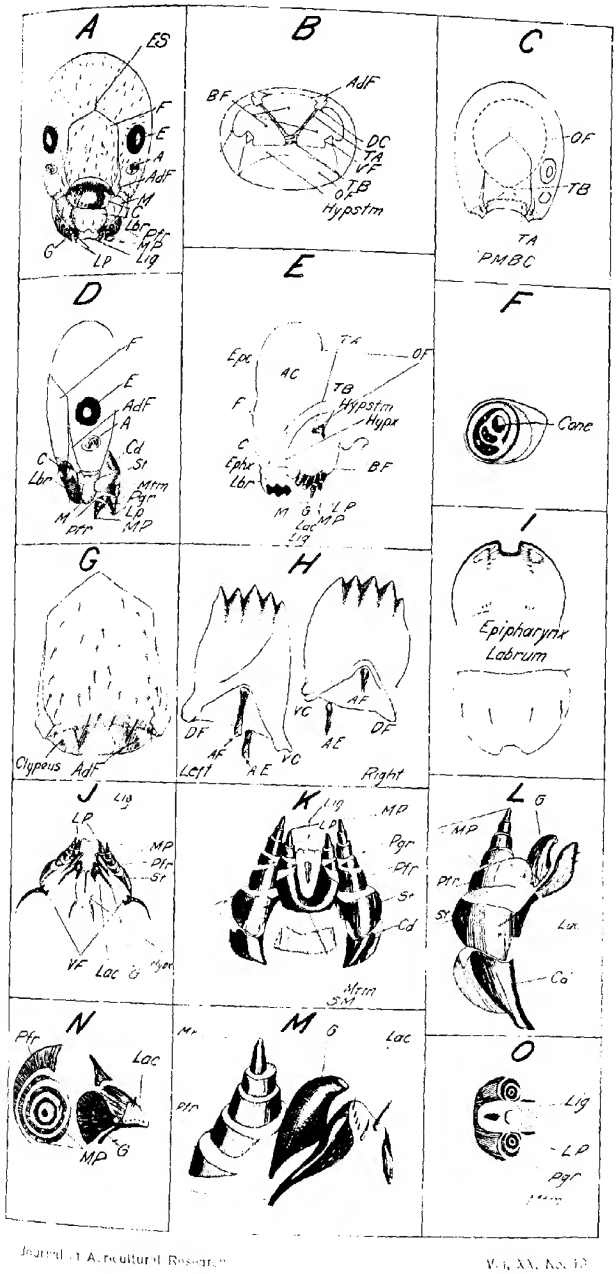
PLATE 90

Neodiprion lecontei: Sixth-stage larva.

A.—Front view of head.
B.—Ventral (or apical) view of head capsule.
C.—Front view of head capsule.
D.—Lateral view of head.
E.—Sagittal section of head.
F.—Antenna.
G.—Frons, adfrons, and clypeus.
H.—Mandibles.
I.—Epipharynx and labrum.
J.—Internal view of hypopharynx, maxillæ, and labium.
K.—External view of maxillæ and labium.
L.—External view of maxillæ.
M.—Interior and apical view of maxilla.
N.—End view of maxilla.
O.—End view of labium.

EXPLANATION OF SYMBOLS

A, antenna.
AC, alimentary canal.
AdF, adfrons.
AE, attachment of extensor muscle.
AF, attachment of flexor muscle.
BF, buccal foramen.
C, clypeus.
Cd, cardo.
DC, dorsal or anterior condyle for mandible.
DF, dorsal or anterior fossa of mandible.
E, eye.
Epc, epicranium.
Ephx, epipharynx.
ES, epicranial suture.
F, frons.
G, galea.
Hypstm, hypostoma.
Hypx, hypopharynx.
Lac, lacinia.
Lbr, labrum.
Lig, ligula.
LP, labial palpi.
M, mandible.
MP, maxillary palpi.
Mtm, mentum.
OF, occipital foramen.
Pfr, palpifer.
Pgr, palpiger.
Plstm, pleurostoma.
PMBC, posterior margin of buccal cavity.
Sm, submentum.
St, stipes.
TA, tentorial arms.
TB, tentorial bridge.
VC, ventral or posterior condyle of mandible.
VF, ventral or posterior fossa for mandible.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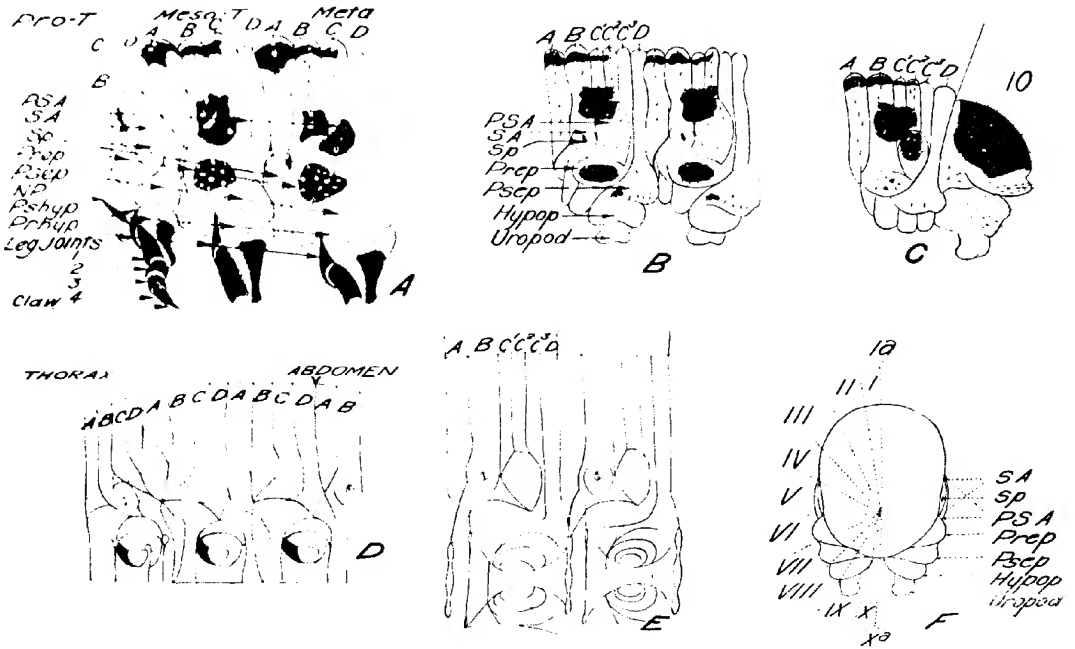
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THORAX
ABDOMEN
D
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I
II
III
IV
V
VI
VII
VIII
IX
X
Xa
F

PLATE 91

Neodiprion lecontei: Sixth-stage larva.

A.—External view of the thorax.

B.—External view of the second and third abdominal segments.

C.—External view of the ninth and tenth abdominal segments.

D.—Internal view of thoracic skin.

E.—Internal view of the skin of the second and third abdominal segments.

F.—Diagrammatic cross section of the abdomen showing the longitudinal areas of the body on its transverse circumference.

EXPLANATION OF SYMBOLS

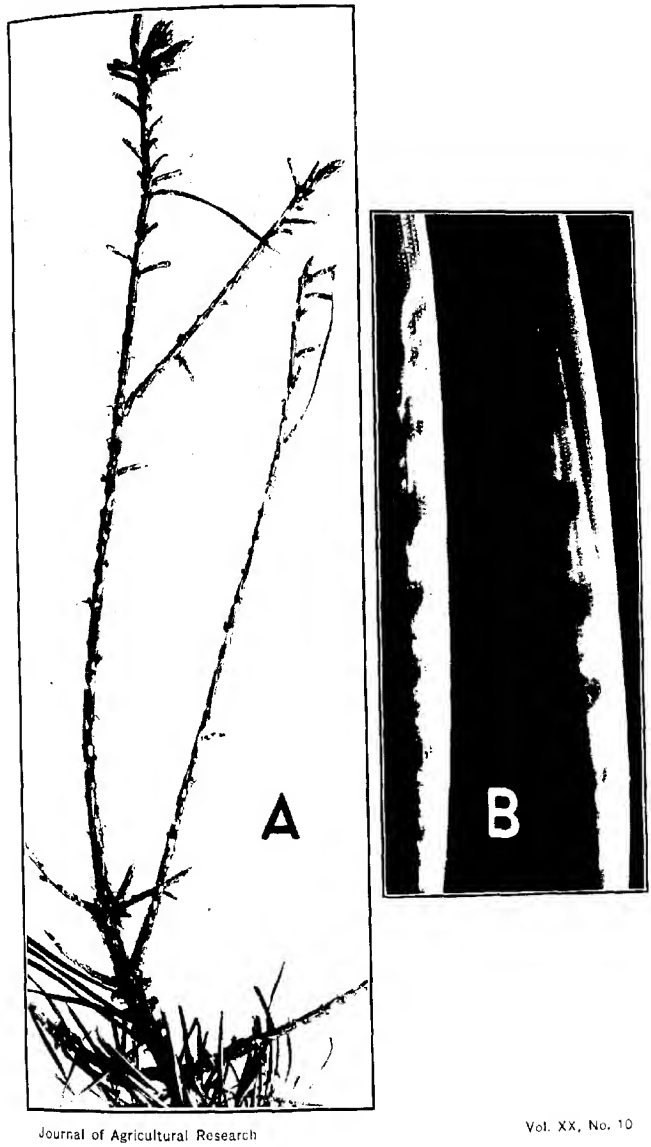
Hypop, hypopleurite.
NP, neck plate.
Prep, preepipleurite.
Prhyp, prehypopleurite.
PSA, postspiracular area.
Psep, postepipleurite.
Pshyp, posthypopleurite.
SA, spiracular area.
Sp, spiracle.

I^a, middorsal; I, dorsal; II, subdorsal; III, laterodorsal; IV, supraspiracular; V, spiracular; VI, epipleural; VII, pleural; VIII, hypopleural or lateroventral; IX, adventral; X, ventral; and X^a, midventral.

PLATE 92

Neodiprion lecontei:

A.—Some defoliated twigs showing feeding on bark of stem.
B.—Eggs within needles of *Pinus virginiana*.


A
B

AMYLASE OF RHIZOPUS TRITICI, WITH A CONSIDERATION OF ITS SECRETION AND ACTION

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INTRODUCTION

That certain mold fungi secrete amyclastic and other enzyms has been known for a long time. However, much of the work in this direction has been centered around a few common forms, especially in the genera Aspergillus and Penicillium. In fact, the same organism has been selected by many investigators who studied the same or different phases of enzymic production. The literature on the subject is already very large and has been reviewed and listed in many of the publications of recent years. For this reason the writer will refer only to such articles in the body of the paper as are germane to the particular subject under discussion.

Rhizopus tritici was used for this investigation because it is responsible for large losses of sweet potatoes and other vegetables under storage and transportation conditions. Its parasitism has been proved repeatedly by inoculations into sweet potatoes, where it causes a rot identical in appearance with that produced by *R. nigricans*. Preliminary experiments were made with *R. nigricans*, which showed that it produces amylase in abundance. No attempt has been made to duplicate with *R. nigricans* the experiments carried out with *R. tritici*. So far as the writer is aware these are the first experiments of the kind conducted with *R. tritici*.

Some of the work of other investigators has been duplicated as far as the method employed would permit, the purpose being to compare *Rhizopus tritici* with some of the fungi hitherto studied. Some of the results of previous investigators were corroborated, while others were not, which indicates that no sweeping generalizations regarding all fungi can be drawn from the study of a single organism.

METHOD OF EXPERIMENTATION

The investigations were carried out mostly with the powdered mycelium, although the diffusion of the enzym into the culture solution was not entirely disregarded. For certain phases of the work extracts of the mycelium were used. The fungus was grown on a modified Czapek's nutrient solution or on sweet potato bouillon for most of the comparative

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studies. For some parts of the work Czapek's nutrient solution was preferable, since it was then possible to cultivate the fungus in a substrate of known composition. On the other hand, the fungus made a luxuriant growth on sweet potato bouillon, and for experiments, such as the influence of temperature on secretion, this medium was usually employed.

The fungus was grown in 2-liter Erlenmeyer flasks containing about 750 cc. of the sterile solution, on which enough fungous felt was produced to carry out several comparative experiments.

Preliminary experiments showed that the fungus grew poorly on a solution with sodium nitrate and cane sugar as a source of nitrogen and carbon, respectively. Ammonium nitrate was therefore substituted for sodium nitrate and glucose or potato starch, or both, for cane sugar in Czapek's nutrient solution. The composition of the solution as finally prepared is as follows:

Water	1,000.00 cc.
Magnesium sulphate (crystals)	.50 gm.
Potassium acid phosphate	1.00 gm.
Potassium chlorid	.50 gm.
Ferrous sulphate	.01 gm.
Ammonium nitrate	5.00 gm.
Glucose, starch paste, or both, in varying amounts to suit the requirements of the experiments as a source of carbon.	

The sweet potato bouillon is prepared as follows: To the peeled potatoes add double the weight of water; steam for one hour, then squeeze out the liquid through gauze; steam a second time, filter into flasks, and autoclave for 20 minutes at 13 pounds pressure. The sweet potato bouillon always contains a considerable quantity of reducing sugar and starch paste.

Rhizopus tritici grew well on both of these solutions and produced a thick, heavy felt in from 7 to 10 days at a temperature of 25° to 35° C. The better growth was made on the sweet potato bouillon. Contrary to what might be expected, starch paste was more efficient as a source of carbon in Czapek's modified nutrient solution than glucose. The organism was grown in incubators, the temperatures of which did not fluctuate more than 1°.

At the end of the growth period the mycelium, which formed a thick felt on the surface of the medium, was removed and washed in running water for about 15 minutes. It was treated subsequently according to Dox's (*9*) [1] modification of Albert and Buchner's "acetondauerhefe" method. After washing, the mycelium was stirred constantly in an excess of acetone for 10 minutes, squeezed as dry as possible, and treated a second time for 2 minutes in a fresh supply. This acetone was removed as in the former case, and the mycelium was treated with ether for 3

[1] Reference is made by number (italic) to "Literature cited," p. 784–786.

minutes. When air-dry the mycelium was put into small flasks and held at a temperature of 9° C. until required for use. Experiments to be discussed later will show that the mycelium can be held at 9° or even higher for several months without any appreciable loss in its ability to hydrolyze starch.

The hydrolysis by the mycelium or extract was carried out in 150-cc. pyrex flasks. A weighed portion of the mycelium was ground in fine quartz sand and transferred to the flasks, to which was added a measured quantity of the starch paste solution made in distilled water. While the percentage of starch is not material, a 0.5 per cent solution was used for most of the work. After the addition of 2 cc. of toluol to each flask as an antiseptic it was plugged by a cork with a small groove at the side to allow for the escape of the expanded air when steamed at the close of the experiment. Hydrolysis was carried out at different temperatures, the results of which are shown elsewhere.

C. P. chemicals were used in the preparation of the culture media. The Irish potato starch was obtained from Eimer and Amend. The sweet potato starch was prepared by the writer. Preliminary experiments showed that neither contained any reducing sugars. The sand used for grinding the mycelium was purified by washing in distilled water and then burning for an hour or more in a crucible. The water in which sand so prepared was suspended did not reduce copper.

At the close of the digestion period the enzym was inactivated by steaming the flasks in an Arnold steam sterilizer for about 15 minutes. To avoid evaporation during the process of heating, oiled paper was fastened with a rubber band over the cork and around the neck of the flask. Before this method was finally adopted tests were made to determine the temperature reached in a given volume of solution in a given length of time. Table I shows the results of these tests, made with tap water in Erlenmeyer flasks, with an initial temperature of 14° to 15° C. There was a small slit at the side of the cork to allow for expansion, and a thermometer was run through it, with the bulb submerged in the water.

TABLE I.—*Temperature reached by a certain volume of water when heated a given length of time in an Arnold sterilizer (average of several tests)*

Volume of water.	Capacity of flask.	Temperature.	Time.
Cc.	*Cc.*	*°C.*	*Minutes.*
50	100	80.5	1
50	100	93.0	2
100	100	69.0	1
100	100	89.5	2
100	100	96.0	3
500	500	65.0	2
500	500	79.5	3

The loss of water by the use of the method described above was less than 0.1 gm. in a flask of 150-cc. capacity containing 100 cc. of solution.

After the flasks had been heated for 15 minutes the contents were filtered through a fine quality of absorbent cotton to remove the mycelium and sand. Filter paper was first tried but was finally rejected in favor of the cotton for two reasons: (1) The solution filtered slowly, thereby introducing considerable error as a result of evaporation; (2) it removed much of the nonhydrolyzed starch. After the filtrate cooled, the reducing sugars were determined volumetrically, according to the method of Clark (*8*). This is a quick and accurate method for the determination of small amounts of reducing sugars by titrating the reduced copper without removing it from the residual copper solution.

The results of starch hydrolysis set forth in the discussion of the following experimental data are expressed in milligrams of reducing sugars in a given volume of solution or in total reducing sugars formed. The results are expressed mostly in milligrams per 10 cc., because 10 cc. of solution are usually employed in making the titrations. If the quantity of reducing sugars in 10 cc. of solution is known, the total reduction or that portion of the starch remaining nonhydrolyzed can be calculated.

It is evident from the method employed that no account is taken of products intermediate between the starch and reducing sugars. It is likely that such products, for example dextrins, are formed in all cases, but the determination of the reducing sugar meets the requirements of the problem in hand, which has for its object mainly to show that a vigorous starch-splitting enzym is formed by *Rhizopus tritici*, and also some of the conditions upon which the production of this enzym depends and how certain environmental factors may influence its activity.

Various modifications of these methods were used in certain of the experiments, but such changes in the methods required to meet the needs of the experiments will be explained in sufficient detail when the results of the experiments are presented and discussed.

It was shown by Dox (*9*) that a considerable autolysis of the fungus mycelium actually takes place. In some enzym experiments where hydrolysis is measured by the amount of reducing sugars formed, a considerable error is likely to be introduced if a correction is not made for the autolysis of the mycelium itself. A number of tests have shown that the amount of autolysis produced from 0.25 gm. of mycelium suspended in 50 cc. of distilled water varies from 1.20 to 7.39 mgm. per 10 cc., with an average of 6.38 mgm. Where a considerable amount of reduction of the starch is involved, this amount would not introduce a very considerable error. On the other hand, where the total hydrolysis is small a considerable error in the final results might be introduced. In all experiments, except where the results would not be influenced one way or the other, the autolysis of the mold was determined and deducted from the total reducing sugars formed in the system.

EXPERIMENTAL DATA

HYDROLYSIS OF RAW STARCH

Preliminary experiments showed that *Rhizopus tritici* produced an enzym which hydrolyzed starch to reducing sugars. This fungus is commonly found as a cause of the decay of sweet potatoes in storage and along with *R. nigricans* probably is responsible for the greater percentage of decay attributed to the Mucoraceæ. In just what form they utilize carbohydrates when growing on the sweet potato is not known, but that they are responsible for certain carbohydrate changes in the host directly through their own activity or by stimulating the host to do so, or both, will be shown by investigations now under way.

Most of the previous work with amylase secreted by fungi was carried out with starch paste or soluble starch. This obviously is not the form in which it occurs in the host, and although the enzym might digest starch paste, it is not safe to conclude that it would act on raw starch, or if at all, to the same degree.

Ward (*24*) concluded from the appearances of the starch grains of the Irish potato that they were not acted on by Pythium, while Hawkins and Harvey (*14*), on the other hand, found from a chemical determination of the total starch present in the sound and rotted portions of the same potato that the starch content was actually lower in the latter than in the former. That all fungi do not behave the same as regards their action on starch is evident from the fact that Hawkins (*13*) found that neither *Fusarium oxysporum* nor *F. radicicola* apparently alters the starch content of Irish potato. It is evident from the results of the authors just cited that no general conclusions can be drawn for all fungi from the behavior of any one or more fungi. The first experiments, therefore, were designed to test the comparative hydrolysis of raw starch and starch paste. The results are given in Table II.

TABLE II.—*Results of hydrolysis of raw starch expressed in terms of reducing sugar (average of several tests)*

Mycelium.	Water.	Starch.	Time of hydrolysis.	Temperature.	Hydrolysis in milligrams per 10 cc. of solution.	Total hydrolysis.	Source of starch.
Gm.	*Cc.*	*Per cent.*	*Hours.*	*° C.*		*Mgm.*	
0.10.....	50	0.5	5.0	27.5	1.06	5.30	Sweet potato.
.20.....	100	.5	18.0	27.5	5.945	59.45	Do.
.20.....	100	.5	17.5	27.5	7.38	73.80	Do.
.20.....	100	.5	17.5	27.5	7.07	70.70	Do.[a]
.20.....	100	.5	17.5	27.5	6.28	62.80	Irish potato.
.20.....	100	.5	17.5	27.5	9.12	91.20	Do.[a]

[a] Starch macerated in sand before hydrolysis was started.

While an examination of Table II shows that both raw Irish and sweet potato starch are hydrolyzed, no large amount of invert sugars are produced after hydrolyzation is carried on for 17.5 to 18 hours. Grinding the starch in fine quartz sand does not seem to influence the amount of hydrolysis appreciably.

That starch paste is more readily hydrolyzed than raw starch is evident from the results of the following experiments. Two sets of flasks were prepared to contain 0.2 gm. of powdered mycelium. To one set were added 100 cc. of sterile distilled water and 0.5 gm. of raw sweet potato starch, and to the other 100 cc. of water containing 0.5 gm. of starch paste. A third set contained 100 cc. of water and 0.2 gm. of mycelium but no starch. Toluol was added as an antiseptic. Hydrolysis was carried on for 18 hours at 40° C. In the set with water and mycelium 1.98 mgm. of reducing sugar, representing autolysis of the fungus, were found per 10 cc. of solution. This amount of reducing sugar was deducted from the results obtained from the other two sets. Reducing sugars equivalent to an average of 1.20 mgm. per 10 cc. of solution were obtained from the raw starch, while 27.95 mgm. were obtained per 10 cc. from the starch paste solution, or an amount more than 23 times as large.

INFLUENCE OF AGE OF MYCELIUM ON POWER OF HYDROLYSIS

To carry out any considerable number of comparative experiments at different times the mycelium must be produced in quantity and kept for some time. Before such material could be used for comparative studies it was necessary to determine whether the mycelium lost its power of digestion with age, and if so to what extent.

The mycelium was grown in large flasks on sweet potato bouillon. At the end of 8 days' growth it was removed and prepared according to the method already described. Hydrolysis was carried out at different times at a temperature of 27.5° C. for 19 hours by the use of 0.25 gm. of powdered mycelium. A starch paste solution was prepared which contained 53.4 mgm. of starch per 10 cc. of solution. This sterilized starch solution was tightly stoppered to prevent evaporation and contamination and was stored at a temperature of 9°. Two days after the mycelium was collected the first experiment was conducted. Fifty cc. of the starch paste and 0.25 gm. of the mycelium finely ground in sand were used in 150-cc. pyrex flasks, with 2 cc. toluol added as an antiseptic. Two flasks with mycelium and starch paste and one control flask containing mycelium and 50 cc. of water were used in each test. The amount of autolysis was deducted from the average of two closely agreeing samples. The results appear in Table III.

There was a slight decrease in the amount of reducing sugars in the tests of the last three months. From the results it seems safe to conclude that the mycelium may be kept for several months without any appreciable

loss in reducing power. These results are in accord with those of Dox (*9*), who found that mycelium may be kept almost indefinitely without losing its activity.

TABLE III.—*Amount of reducing sugars produced by the same samples of mycelium used at different times*

Feb. 20.	Mar. 5.	Mar. 18.	May 7.	June 10.	June 25.	Sept. 26.
Mgm. 216.337	*Mgm.* 225.5	*Mgm.* 222.65	*Mgm.* 233.1	*Mgm.* 208.55	*Mgm.* 205.4	*Mgm.* 204.9

INFLUENCE OF DIFFERENT TEMPERATURES ON THE AMYLOCLASTIC ACTIVITY OF THE MYCELIUM

Although it was shown by Table III that mycelium may be stored at 27.5° C. for a number of months without materially affecting the activity of the enzym, it can not be concluded that it can be kept unimpaired at any temperature. As a matter of fact, the following results show that the hydrolytic power of the enzym is somewhat impaired when held for a time at a high temperature.

The mycelium for these experiments was produced in six 2-liter flasks containing about 750 cc. of sweet potato bouillon. At the end of the growth period the mycelium was made into one composite sample and held at a temperature of 9° C. for 18 hours. A sample was then removed and its original hydrolytic power was determined. The remainder was divided into three lots, one being stored at 9°, one at 35°, and one at 60°.

To determine the original hydrolytic power of the mycelium two 0.25-gm. lots were weighed out and ground in fine quartz sand. To one flask containing enzym powder were added 100 cc. of a 0.5 per cent starch paste solution and to the other 100 cc. of sterile distilled water. After the addition of toluol as an antiseptic both were digested for 18 hours at 40° C. In the former 2.2 mgm. and in the latter 33.46 mgm. of reducing sugars were found in 10 cc. of solution, or a total of 22 mgm. and 334.6 mgm. in 100 cc., respectively. These figures will serve as a basis for comparison of future tests of the same lot of mycelium stored at different temperatures. (Table IV.)

TABLE IV.—*Amount of starch hydrolized by mycelium stored at different temperatures for a given length of time*

[Expressed in milligrams per 10 cc. of solution]

Temperature.	Original sample before storage.	After 12 days' storage.	After 39 days' storage.	After 73 days' storage.
°C.				
9	33.46	29.649	39.429	37.448
35	.....	35.148	37.740	30.100
60	.....	22.428	21.400	17.000

The results show that the hydrolytic power of the mycelium stored at 60° C. at the end of 73 days is somewhat more impaired than that of mycelium stored at 35° and 9° for the same length of time. On the other hand, the results indicate that the mycelium may be safely stored for a considerable time at 9° and 35° without materially affecting the enzym.

EFFECT OF TEMPERATURE ON THE HYDROLYTIC POWER OF THE ENZYM

It is generally understood that enzyms are more resistant to heat when in the form of a powder than when in suspension. Kjeldahl (*18*) found that the action of amylase at 0° C. was very slow but increased rapidly with the increase in temperature up to 60° and at 70° became insignificant. Similar results were obtained by Durandard (*11*), who reports that the optimum temperature for the hydrolysis of rice starch by an extract of *Rhizopus nigricans* to be 45°. He obtained some hydrolysis at 10° and four times as much at 45° as at 30°. It diminishes rapidly toward 55°, becoming very feeble at 60° and nothing at 70°. The writer found likewise the optimum temperature for the hydrolysis of potato starch to be about 45°, with a gradual decrease above that temperature, becoming practically nothing at 60°. Effront (*12*) concludes also that the temperature has no other effect than to reduce the diastatic power, and the nearer the temperature approaches 70° the greater is the reduction. White (*26*) found that certain enzyms in dry oats, among them diastase, were not injured on heating for 4½ hours to 100°, but that an exposure for one hour at 130° did destroy the ferments.

That the amylase contained in *Rhizopus tritici* is destroyed at a temperature of 60° C. is shown in the following experiments. Five-tenths gm. of mycelium was extracted for 24 hours in each of two flasks containing 150 cc. of sterile distilled water at a temperature of 9°. The contents of the flasks were then filtered, and 100 cc. were pipetted into 250-cc. flasks. Both flasks were exposed for an additional 100 hours, one at a temperature of 60° and one at 9°. The contents of each flask were then diluted with 100 cc. of a 1 per cent starch paste and hydrolyzed for 18 hours more at 40°. At 60° and 9° the reducing sugars formed per 10 cc. of solution were on an average 1.36 and 36.36 mgm., respectively. Although a little reducing sugar was formed, it is believed that it was derived by autolysis of the mycelium during the period of extraction.

INFLUENCE OF GLUCOSE ON THE HYDROLYSIS OF STARCH

The stimulating and retarding effect of certain substances, especially those identical with or similar to the products of hydrolysis, have been subjects of investigations for a long time. Hill (*15*) found that glucose interfered with the action of maltose, and Armstrong (*1*) pointed out a number of cases where the reaction products inhibited the action of the

enzyms. Kellerman (17) found that the alkalies without exception seemed to be detrimental and the metals generally injurious to the action of Taka diastase. From the results obtained by these and other investigations it is evident that many substances influence the rate of action of the enzym. The data shown here are the results of a single experiment. Four flasks marked a, b, c, d were prepared, each to contain 0.25 gm. of powdered mycelium. A second lot of flasks was prepared, and into flask a were added 100 cc. of a 0.5 per cent starch paste solution; into flask b 125 cc. of a 0.5 per cent starch paste and 0.625 gm. glucose; into flask c 125 cc. of a 0.5 per cent starch paste and 2.5 gm. glucose; into flask d 125 cc. of a 0.5 per cent starch paste and 6.25 gm. glucose. After thorough mixing, 25 cc. were drawn from flasks b, c, and d, and the reducing sugars were determined volumetrically. The contents of flasks b, c, and d were then poured into the corresponding flasks containing mycelium and digested for 18 hours at 40° C., with the results given in Table V.

TABLE V.—*Amount of reducing sugars before and after hydrolysis*

[Expressed in milligrams per 10 cc. of solution]

Sample.	Reducing sugars originally present.	Reducing sugars present at end of the digestion period.	Increase in reducing sugars.
a	0	42.476	42.476
b	53.040	86.899	33.859
c	181.580	216.080	34.500
d	438.386	472.108	33.722

It seems evident from the results of a single test that the presence of glucose decreases the activity of the amylase, since the total reducing sugars formed in sample a is considerably greater than in samples b, c, and d. On the other hand, the closely agreeing results of b, c, and d indicate that the amount of glucose present at the strength used in this experiment has no effect upon the hydrolysis of the starch.

RELATION OF QUANTITY OF STARCH PRESENT TO AMOUNT OF HYDROLYSIS

This subject naturally involves a consideration of the law of "mass action," and in the literature on this subject there appears to be no agreement of opinion on the question. The investigations show that so far as enzyms are concerned so many factors influence the reaction that no definite conclusion can be drawn. For example, Brown and Glendinning (4) showed that when the concentration of the enzym relative to the starch in the early stages is very small, the amount of starch hydrolyzed per unit volume will be very large compared with the amount of the combination of starch and enzym. If the concentration of the unchanged substrate remains very large in relation to that of the

combination, the latter will remain nearly constant in amount and equal amounts of starch will be hydrolyzed in equal times, the curve being a straight line. On the other hand, when the concentration of the starch has been greatly reduced, the amount of the combination and accordingly the hydrolysis will follow more closely the law of "mass action." Similar results were obtained by Armstrong working with lactose, maltose, and emulsin. Other investigators have found various factors influencing the reaction between the enzym concerned and the substrate. For a full consideration of the theory involved in "mass action" the reader is referred to a discussion of the subject by Bayliss (*2*).

The data submitted in Table VI are the results of a considerable number of experiments which were varied to suit the requirements of the problem. In the first series of experiments the amount of enzym power (0.25 gm.) was constant and the volume of the starch paste solution was varied. The time of hydrolysis was 19 hours at 32° C.

TABLE VI.—*Total amount of reducing sugars and reducing sugars per 10 cc. of solution in different volumes of a 0.5 per cent starch paste solution*

Sample.	Volume of solution.	Reducing sugars per 10 cc.	Total reducing sugars.
	Cc.	*Mgm.*	*Mgm.*
a	50	39.984	199.92
b	100	25.864	258.64
c	150	26.600	399.00
d	200	16.400	328.00

In sample a the reducing sugars per 10 cc. is considerably larger than in sample d, while b and c are about the same. In total reducing sugars found there is a progressive increase up to and including 150 cc., and then a slight decrease. While in sample a some starch yet remained nonhydrolyzed, it is likely that on approaching the end point the rate of hydrolysis was slowed up. It is probable that a shorter period of hydrolysis would have given a different curve and that the total reducing sugars formed would have paralleled the reducing sugars per 10 cc.

Somewhat similar results were obtained when the total volume of solution (100 cc.) and the amount of enzym powder (0.25 gm.) were constant but the quantity of starch paste was varied. A 1.5 per cent starch paste solution was used in the dilutions, enough distilled water being added to make a total volume of 100 cc.

The time of hydrolysis was 19 hours at 32° C. The average results of parallel tests are shown in Table VII.

The results show an increase in reducing sugars with the increase in the amount of starch present from sample a to sample c, inclusive, and then a slight decrease. In sample a, although the end point had been more closely approached than in any of the other samples, some starch still remained unhydrolyzed. If it were not for the results obtained in samples d

and e, it might be assumed that the accumulation of reducing sugars acted as a paralyzer to further action of the enzym or, as has been suggested by some investigators, the enzym entered into combination with the products of the hydrolysis and consequently became inactive.

TABLE VII.—*Amount of 1.5 per cent starch paste used, total reducing sugars, and reducing sugars per 10 cc.*

Sample.	Total volume of solution.	Volume of starch paste.	Reducing sugars per 10 cc.	Total reducing sugars.
	Cc.	*Cc.*	*Mgm.*	*Mgm.*
a	100	20	21.240	21.24
b	100	40	35.632	356.32
c	100	60	37.842	378.42
d	100	80	33.498	334.98
e	100	100	32.551	325.51
f	100	00	5.198	51.98

In the series of experiments reported in Table VIII different amounts of a 1 per cent starch paste solution were used, and enough water was added to make a total volume of 500 cc. One-fourth gm. of enzym powder was added to each set of flasks. The time of hydrolysis was 18 hours at 40° C.

TABLE VIII.—*Amount of 1 per cent starch paste used, total reducing sugars, and reducing sugars per 10 cc.*

Sample.	Total volume of solution.	Volume of starch paste.	Reducing sugars per 10 cc. of solution.	Total reducing sugars.
	Cc.	*Cc.*	*Mgm.*	*Mgm.*
a	500	20	3.1850	159.250
b	500	50	6.3050	315.250
c	500	100	8.9375	446.875
d	500	200	10.4000	520.000
e	500	300	11.9600	598.000
f	500	400	12.1550	607.750
g	500	500	8.1575	407.875

The amount of reducing sugars per 10 cc. increases with the increase in the amount of starch from sample a to sample f and then decreases. An approach toward the end point might here also account for the lesser amount of hydrolysis in the more dilute solutions if the total reduction in sample g, which contains the largest amount of starch, was not actually less than in several of the other samples.

A final series of experiments was carried out in which the total volume of 0.5 per cent starch paste was varied but the amount of enzym powder (0.25 gm.) was constant. Hydrolysis was carried on for 18 hours at 40° C. (Table IX.)

There was a decrease in the reducing sugars per 10 cc. and an increase in total sugars as the volume of the solution increased from sample a to sample e, and then a reverse of the process.

TABLE IX.—*Volume of 0.5 per cent starch paste solution used, total reducing sugars, and reducing sugars per 10 cc.*

Sample.	Volume of starch paste.	Reducing sugars per 10 cc.	Total reducing sugars.
	Cc.	*Mgm.*	*Mgm.*
a	50	45.16	225.8
b	100	37.64	376.4
c	200	28.28	565.6
d	300	19.74	592.2
e	400	14.42	576.8
f	500	9.80	490.0

IS AN END POINT IN HYDROLYSIS REACHED?

Theoretically an end point should not be reached without shifting the point of equilibrium of the solution. As a matter of fact, to settle the question is difficult by any method, since there may be intermediate products between starch and reducing sugars which are not revealed by the iodin test and do not reduce copper. The experiments were made with extracts of the mycelium. The mycelium (1.5 gm.) after powdering was extracted in a pyrex flask for 24 hours at 9° C. in 300 cc. of distilled water. The extract was then filtered. Two hundred fifty cc. of the extract were then diluted with 250 cc. of a 2 per cent starch paste solution. After thorough mixing 20 cc. were drawn off, 2.5 cc. concentrated hydrochloric acid were added and the mixture was hydrolyzed by boiling for 2.5 hours. The solution was neutralized with sodium hydroxid made up to 200 cc. with water, and the starch present was determined as reducing sugars. A preliminary test showed that no reducing sugars were present in the original starch paste solution. After hydrolysis reducing sugars equivalent to 104 mgm. of starch per 10 cc. were found.

The solutions were mixed on May 22 and hydrolysis carried out at 45° C. Reducing sugars were determined approximately 24 hours apart for several days thereafter with the results shown in the Table X.

TABLE X.—*Amount of reducing sugars at different dates and equivalent in starch*

[Expressed in milligrams per 10 cc.]

Date.	Reducing sugars.	Equivalent in starch.
May 23	56.516	52.560
24, 9.30 a. m	79.230	73.689
24, 3.30 p. m	84.518	78.602
26	99.968	92.970
27	103.092	95.875
28	105.364	97.988
29	108.866	101.245
30	108.866	101.245
31	108.866	101.245
June 9	108.889	101.267

The results show that the amount of reducing sugars steadily increased for the first 7 days but remained practically stationary thereafter. At the end of 18 days a small amount of starch yet remained nonhydrolyzed.

To determine whether the addition of a small amount of starch would stimulate further hydrolysis, 100 cc. of the solution described on page 772 were mixed with 100 cc. of an approximately 0.5 per cent starch paste solution. A small amount (20 cc.) was drawn off, and the actual amount of starch was determined. The remainder was hydrolyzed at 45° C.

After acid hydrolysis reducing sugars to the amount of 1,568 mgm. were found in 200 cc. of the original solution. Of this amount 1,088.89 mgm. of reducing sugars and 27.33 mgm. of nonhydrolyzed starch (equivalent to 29.38 mgm. reducing sugars) were brought over to the solution when the dilution was made, making a total of 1,118.27 mgm. reducing sugars. Deducting this amount from the amount originally found (1,568 less 1,118.27 mgm.), the result gives the amount of reducing sugars added in the form of starch, or 449.73 mgm. This is calculated to be equivalent to 418.2489 mgm. of starch. To this amount should be added 27.33 mgm., the quantity of nonhydrolyzed starch present before the solutions were mixed, making a total of 445.58 mgm. starch present in 200 cc. of the solution when hydrolysis was started. After hydrolysis had gone on for 24 hours a sample was taken, and the reducing sugars were determined, which gave in 200 cc. a total of 1,516.6 mgm. There was no starch left in the solution according to the iodin test. Since in the original solution there were 1,568 mgm. of reducing sugars present, 51.4 mgm. (equivalent to 47.8 mgm. starch) remain unaccounted for, except as intermediate products between starch and reducing sugars.

Parallel experiments, which will not be given in detail, gave similar results.

The evidence brought out shows that an equilibrium is established in the solution before quite all the starch is hydrolyzed. Also that if more starch is added and the solution is diluted the starch finally disappears so far as its presence is indicated by the iodin test.

So far as these and many other results go, an end point is reached if the disappearance of the starch alone is considered. Viewed from the standpoint of reducing sugars found, an end point is not reached. Many experiments not designed primarily to demonstrate this point have shown that no starch, as indicated by iodin, remains in the solution after a definite length of time. On the other hand, starch is shown to be present in some solutions by the same test after a considerable time. It was also shown by experiments that if an end point was not reached at a certain temperature, namely 45° C., the starch would completely disappear in 24 hours by shifting the solution to a temperature of 35°.

Perhaps an explanation of some of these facts may be found in the results of other investigations. The results of the above experiments show that all the starch was not accounted for as reducing sugars,

although in such solutions no starch was present, if judged by the iodin test. This difference might be explained by the presence of dextrins as intermediate products. Brown (3) claims that in the action of diastase on starch the reaction ends when the composition of the product is 80.8 per cent maltose and 19.2 per cent dextrin. Maquenne and Roux (21), however, suggest that the equilibrium of 80.8 per cent maltose and 19.2 per cent dextrin referred to above is due to insufficient activity of the enzym and that if malt diastase is activated by acid in small amount the whole of the starch is found to be converted into sugar, so no dextrins remain. Bayliss (2) found that the amount of maltose produced in the first stage was greater than the equilibrium position of Brown and Heron because it was allowed to proceed for a longer time.

Although the writer did not use a temperature above 40° C., this temperature might have had some bearing on the proportion of sugar to dextrins, in accordance with the interesting results of Brown and Heron (5).

These investigators found that the dextrinase is more injured by a temperature of 68° C. than the amylase. According to this theory they explain the fact that when starch paste is acted on by diastase which has been exposed to a temperature of 68° there is less maltose and more dextrin formed than when the enzym has not been so heated. This raises the question as to just where the influence of temperature makes itself felt. Furthermore, facts which might bear upon the question were brought out by Tammann (23), who reports that an increase of hydrolysis was obtained in a stationary system by altering any of the other conditions of the equilibrium, such as the addition of more amygdalin, renewal of the products of the reaction, raising the temperature, or increasing the dilution. In Tammann's work the retardation would virtually be due to the accumulation of the products of the reaction.

GROWTH AND HYDROLYSIS IN A SOLUTION OF STARCH PASTE

The remarkable power of *Rhizopus tritici* to grow on almost any kind of medium is evident when we consider that it can be isolated from a great variety of decayed substances. Its ability to hydrolyze starch in a solution poor in nutrient material was tested several times by inoculating a starch paste solution made with distilled water. While such a solution would contain nutrient substances in addition to the carbohydrates introduced in the form of starch, a considerable growth would hardly be expected, but, nevertheless, a fair growth was made and hydrolysis of the starch went on.

The experiments were made in Erlenmeyer flasks containing 500 cc. of a 0.5 per cent starch paste solution. Some of the inoculations were made with bits of mycelium and spores and some with spores alone. Growth was slow at the outset, the colonies being submerged

at first, a felt forming later on the surface of the liquid. The solutions were tested for reducing sugars at the beginning of the experiments, but in no case were any found. The fungus must then of necessity have either to utilize the starch directly or first have converted it into some simpler form. From time to time some of the liquid was drawn off, and the reducing sugars were determined. The results showed an increasing amount of reducing sugars present with each subsequent determination, from which it is evident that the fungus hydrolyzed the starch in excess of its needs. If the growth continued long enough the solution which was milky in color at first finally became clear, showing that practically all the starch was hydrolyzed. Many experiments in the course of these investigations likewise demonstrated clearly that the fungus hydrolyzed the starch in the solution, although reducing sugars were already present. Furthermore, the hydrolysis of the starch in a solution of starch and glucose began very soon after inoculation, which suggests that the enzym diffuses into the solution soon after the beginning of growth. This subject will receive further consideration in the discussion of an extracellular enzym.

EXTRACELLULAR ENZYM

The results in the following experiments show other interesting facts in addition to the production of an extracellular amylase. Two nutrient solutions a and b, differing in the source of nitrogen, were used. Solution a had the following composition:

Water	1,000.00 cc.
Magnesium sulphate (crystallized)	.50 gm.
Potassium acid phosphate	1.00 gm.
Potassium chlorid	.50 gm.
Ferrous sulphate	.01 gm.
Sodium nitrate	2.00 gm.
Starch	10.00 gm.

Solution b differed from a in that the sodium nitrate was replaced by 5 gm. of ammonium nitrate.

The chemicals were first dissolved in the water by steaming, after which the starch was added and the entire mixture was sterilized by autoclaving.

The growth in these two solutions was remarkably different. In a the mycelium was mostly submerged, while in b a thick felt was formed on the surface. Solution a produced in 16 days of growth a total dry weight of 0.0298 gm.; b, 0.7198 gm., or about 24 times as much. Both solutions were inoculated on October 27. The reducing sugars and starches were determined at stated intervals thereafter, as shown in Table XI.

TABLE XI.—*Amount of reducing sugars and starch present in solutions a and b at stated intervals of time*

[Expressed in milligrams per 10 cc. of solution]

Date.	Solution a.		Solution b.	
	Reducing sugars.	Starch.	Reducing sugars.	Starch.
Oct. 27	0	111.0	0	106.0
29	5.3	103.0	5.8	96.0
31	21.7	83.0	34.6	47.0
Nov. 3	46.0	59.0	35.2	24.0
5	61.4	41.0	21.6	25.0
7	65.3	38.5	14.0	23.8
10	75.0	25.2	7.7	22.8
12	75.8	23.4	6.0	21.6
3 (controls)	0	.....	0	.....
12 (controls)	0	.....	0	.....

From Table XI it is seen that in two days reducing sugars in excess of those used by the fungus were produced with a decrease in the amount of starch. In the a solution the reducing sugars gradually accumulated to the end of the experiment, while the amount of starch decreased, showing that the fungus did not use a corresponding amount of the reducing sugars formed. On the other hand, in solution b the reducing sugars increased up to November 3 and then decreased to the close of the experiment, while the starch decreased rapidly to November 3 and very little thereafter, which suggests that hydrolysis was slowed up as it approached the end point and did not keep pace with the demands of the fungus for reducing sugars. This condition is reflected in the amount of dry matter formed, which is about twenty-four times greater in solution b than in solution a. The amount of starch in the two solutions at the close of the experiment was practically the same. It seems, then, that an extracellular amylase was promptly secreted by the fungus and that it hydrolyzed the starch in excess of the needs of the fungus in one case (a) to the close of the experiment and in the other until November 3, when the reducing sugars consumed exceeded those produced by the hydrolysis of the starch.

Why the difference in the composition of the two solutions plays such a fundamental rôle in the growth of the fungus can not be answered. As previously stated, solution a derives its nitrogen from sodium nitrate and solution b from ammonium nitrate. The growth in the latter case was many times greater than in the former. Since solution a was virtually Czapek's nutrient solution, it was tried at the outset for other work of a similar nature and was later modified by the substitution of ammonium nitrate for sodium nitrate. The solution so modified gave a luxuriant growth of mycelium. Solution a, however, apparently had no inhibitory action on the amylase, so that hydrolysis of the starch went on unhindered.

REMOVAL OF AMYLASE BY FILTERING

The enzym powder was extracted for 24 hours in sterile distilled water. The contents of one set of flasks was filtered through absorbent cotton, which removed the fragments of mycelium, and the others were filtered through four thicknesses of No. 1 Whatman chemically prepared filter paper. A quantity of this filtered extract was then mixed with an equal volume of a 1 per cent starch paste solution and hydrolyzed for 18 hours at 40° C. At the close of the period of hydrolysis the reducing sugars were determined in the usual way. The average of several parallel experiments showed that when filtered through cotton, 172.51 mgm. reducing sugars were formed in 100 cc. of solution but that only 129.32 mgm. were formed when filtered through filter paper.

INFLUENCE OF TEMPERATURE AT WHICH MYCELIUM IS GROWN ON ITS POWER OF HYDROLYSIS

The investigations of the writer and others have shown that the optimum temperature for the activity of amylase is about 45° C. and that activity is reduced by higher and lower temperatures. Since these results, however, were obtained from mycelium grown at one temperature, the question was naturally suggested whether the temperature at which it was grown did not influence the amount of amylase produced. The mycelium was grown on sweet potato bouillon in 2-liter Erlenmeyer flasks. One set of flasks was incubated at 9°, one at 29°, and one at 40°. At the close of the incubation period (10 days) the mycelium was removed from the flasks and treated with acetone and ether in the usual way. The mycelium from the flasks held at the same temperature was made into a compound sample and stored at 9° until used.

The hydrolytic power of the enzym was determined by the use of 0.25 gm. of powder in all tests but two. With the smaller amount of enzym powder hydrolysis was carried out with 50 cc. of a 0.5 per cent starch paste solution; with all others 100 cc. were used. The time of hydrolysis was 18 hours at 40°. At the close of the experiment, the enzym was inactivated by steaming for 10 minutes. The results are given in Table XII.

TABLE XII.—*Results of hydrolysis of starch by mycelium grown at different temperatures*

Temperature.	Milligrams reducing sugars per 10 cc.
°C.	
9	39.700
29	26.854
40	9.933

The results show a very striking influence of the temperature on the production of amylase. A temperature of 40° C. represents about the maximum temperature for growth and 9° the minimum, while a good

growth occurs at 29°. At first thought one might suspect that at the higher temperature the enzym diffuses out into the solution more readily than at the two lower temperatures, and, indeed, one can not say such is not the case. If the hydrolytic capacity of the enzym corresponded to the growth of the fungus in the nutrient solution, as it does not, such a theory might receive strong support. The poorest growth is at the lowest temperature. At 9° the mycelium was mostly submerged, and no fruiting had taken place. On the other hand, at 29° and 40° a thick felt had formed, with some fruiting, though less at 40° than at 29°.

QUANTITATIVE REGULATION OF AMYLASE

The results of many investigations have shown a quantitative regulation of certain enzyms of various fungi. Brunton and MacFayden (7) found that a bacterium produced diastase when cultivated on starch paste but not when grown on meat broth. In the latter case a peptonizing enzym was produced. Pfeffer (*22*) found that in several mold fungi the secretion of diastase depended upon similar conditions, and Brown and Morris (*6*) claim a similar regulatory action with barley, in that when readily assimilable substances were supplied the secretion of diastase did not take place, but when no such substances were available diastase was formed at once. It was likewise found by Wortmann (*27*) that certain molds had the power of excreting a starch-dissolving enzym when starch grains were the only available food and that no secretion took place if sugar or tartaric acid was offered to the organism along with the starch. More recent workers have arrived at similar results with different fungi. Went (*25*) showed that *Monilia sitophila* secreted a number of enzyms, some of which were produced only when the particular substance on which they act was present in the culture solution. Others were produced when substances chemically allied to the products of hydrolysis were present. In general, however, he concluded that the secretion of enzyms was not a hunger phenomenon, since those fungi which were best nourished produced the most enzym. Dox (*9*), on the other hand, demonstrated that for *Penicillium camemberti*, at least, the enzyms were secreted regardless of the chemical nature of the substrate. He found that by cultivating the fungus on a particular substratum the quantity of the corresponding enzym may be increased, but that no enzym not normally produced by the organism could be developed by any special method of nutrition. Katz (*16*) in 1898 published the results of the regulating action of certain chemical substances in the solution of the regulatory secretion of amylase by *P. glaucum*, *Aspergillus niger*, and *Bacillus megatherium* and found that while the amylase secretion was not prohibited by the presence of substances chemically allied to starch, their effect was greatly to inhibit it. He found that the different fungi did not respond exactly in the same way and cites as proof the results with *A. niger* and *P. glaucum*. The presence of sugars in the

solution had a much less inhibitory effect on the production of amylase with *A. niger* than with *P. glaucum*. Similar conclusions were reached by Duclaux (*10*) with *P. glaucum* and *A. glaucus*, though he considered only the enzyms which diffused into the culture medium. The investigations of Kylin (*20*) with *P. glaucum*, *P. biforme*, and *A. niger* corroborate in a general way the results of other investigators. He found no qualitative regulation of the enzyms studied by him (diastase, invertase, and maltase), though a quantitative regulation was conclusively proved. With *P. glaucum* the regulating secretion of diastase was greater than with *A. niger*. Knudson (*19*), on the other hand, demonstrated a qualitative regulation of tannase with *A. niger* and *P.* sp. These fungi produced gallic acid by the fermentation of tannic acid when the latter was added to a modified Czapek's nutrient solution, but if supplemented with sucrose no tannase was formed. A number of other substances as a source of carbon likewise failed to stimulate the secretion of tannase. Young (*28*) studied the inulase formation by *A. niger* in a nutrient solution and found a well-marked quantitative regulation of the production of the enzym. He showed that inulase was produced in greatest amount in the mycelium (extracellular enzyms not studied) when inulin was used as the source of carbon but was likewise produced when other carbohydrates were employed. The substances most closely allied to inulin were most efficient in the production of the enzym.

The results of the writer's experiments which follow demonstrate also a quantitative regulation of amylase in nutrient solutions. Sweet potato bouillon and Czapek's modified nutrient solution (see p. 762) with glucose and starch in combination or alone in varying amounts were used as substrates.

In all these experiments the fungus was grown in 2-liter flasks containing 1,000 cc. of solution. At the end of the growth period the mycelium was removed and prepared in the usual way, according to the "acetondauerhefe" method of Albert and Buchner, the mycelium from the flasks of each series being mixed together to make a compound sample.

EXPERIMENT 1.—The fungus was grown on Czapek's modified nutrient solution with glucose or starch or both as a source of carbon. The cultures were incubated for 8 days at 32° C. Hydrolysis of starch was carried out for 19 hours at 32° by using 0.25 gm. of enzym powder in 50 cc. of a 0.5 per cent starch paste solution. (Table XIII.)

TABLE XIII.—*Source of carbon in Czapek's modified nutrient solution and amount of hydrolysis by the enzym powder per 10 cc. of the substrate*

Series.	Starch.	Glucose.	Reducing sugars.
	Gm.	*Gm.*	*Mgm.*
a	5	5	26. 19
b	0	5	34. 10
c	5	0	39. 28

EXPERIMENT 2.—In this set of experiments sweet potato bouillon was compared with Czapek's modified nutrient solution, the latter containing different amounts of starch and glucose as a source of carbon. The reducing sugars were determined in each series before inoculation and after the fungous growth had been removed, the enzyms in the solutions being inactivated at the end of the growth period by autoclaving the solutions. The cultures were incubated for 10 days at 35° C. The hydrolytic power of the enzym was determined by the use of 0.25 gm. of powder in 100 cc. of a 0.5 per cent starch paste solution. The time of hydrolysis was 18 hours at 40° C. (Table XIV.)

TABLE XIV.—*Source of carbon in Czapek's modified nutrient solution, amount of reducing sugars before and after the growth of the fungus, and the hydrolysis by the enzym powder*

[Expressed in milligrams per 10 cc. of the substrate]

Series.	Starch.	Reducing sugars.		Hydrolysis by enzym powder.
		Before inoculation.	After removal of fungous growth.	
	Gm.			
a	5	0	6.599	13.21
b	0	112.186	40.900	5.16
c	5	112.830	16.882	6.05
d	Not determined [a]	220.570	92.863	24.14

[a] Solutions a, b, and c were Czapek's nutrient solution; d was sweet potato bouillon. The reducing sugar in b and c before inoculation was glucose.

The starch was not determined, but it was shown to be present in series a, c, and d by iodin before the solutions were inoculated. When the fungous growth was removed the starch had all disappeared in series a and d.

From the results it is seen that the largest amount of hydrolysis took place with mycelium grown on sweet potato bouillon (d), where reducing sugars and starch both were originally present. On the other hand there was considerably more hydrolysis with mycelium grown on starch alone as a source of carbon (a) than where glucose was used alone (b) or in combination with starch (c).

The reducing sugars in series b, c, and d were considerably less at the end of the growth period than at the outset, showing that the fungus made use of reducing sugars or had converted them into other substances, possibly alcohol, acids, etc. No starch remained in the solutions. In series a the starch had entirely disappeared, but a small amount of reducing sugar was present. In this case also the fungus had either used a considerable amount of carbohydrate or had converted it into other compounds.

The fungus made the best growth in series d, but it was good in all and fruited abundantly in each of the solutions.

EXPERIMENT 3.—In the following experiments, Czapeck's nutrient solution was used for series a, b, and c, and sweet potato bouillon was used for d, the reducing sugars (glucose in b and c) and starch being determined in the solutions before and after the growth of the fungus. The digestion period was 12 days at 35° C.

In these experiments no account is taken of the amount used by the fungus or that converted to other compounds by it.

The digestive power of the mycelium was determined by using 0.2 gm. enzym powder in 100 cc. of a 0.5 per cent starch paste solution, which was hydrolyzed for 18 hours at 40° C. (Table XV.)

TABLE XV.—*Amount of reducing sugars and starch in solutions before and after the growth of the fungus; also the hydrolysis of starch by enzym powder*

[Expressed in milligrams per 10 cc. of solution]

Series.	Before inoculation.			After removal of fungus.			
	Total reducing sugars after digestion of starch.	Reducing sugars before digestion of starch.	Starch present as reducing sugars.	Total reducing sugars after digestion of starch.	Reducing sugars before digestion of starch.	Starch present as reducing sugars.	Hydrolysis of starch by enzym powder.
a.....	43.62	0	43.62	29.47	10.69	18.78	9.10
b.....	No starch in solution.	112.19	0	.....	36.56	0	.78
c.....	153.60	112.64	40.96	75.15	33.97	41.18	.39
d.....	449.55	272.56	176.99	182.25	144.00	38.25	11.54

These results accord in general with those of the previous experiments, series a and d having the greatest hydrolyzing power and b and c the least.

EXPERIMENT 4.—The foregoing experiment was repeated, the solutions being made to contain roughly the same amount of glucose and starch. The hydrolysis of starch by the enzym powder was determined by using 0.25 gm. enzym powder in 100 cc. of a 0.5 per cent starch paste solution and hydrolyzing 18 hours at 40° C. (Table XVI.)

TABLE XVI.—*Amount of reducing sugars and starch in the solutions before and after the growth of the fungus; also the products of hydrolysis of starch by enzym powder*

[Expressed in milligrams per 10 cc. of solution]

Series.	Before inoculation.			After removal of fungus.			
	Total reducing sugars after digestion of starch.	Reducing sugars before digestion of starch.	Starch as reducing sugars.	Total reducing sugars after digestion of starch.	Reducing sugars before digestion of starch.	Starch as reducing sugars.	Hydrolysis of starch by enzym powder.
a.....	31.275	No sugar used.	31.275	14.30	2.86	11.44	7.15
b.....	No starch.	105.41	0	.....	45.35	0	1.27
c.....	144.680	108.68	36.000	86.32	67.60	18.72	1.24
d.....	524.000	300.37	223.630	107.90	107.90	0	22.94

EXPERIMENT 5.—This experiment was conducted in the same way as experiments 3 and 4. The amyloclastic power of the enzym was determined as in experiment 4. (Table XVII.)

TABLE XVII.—*Amount of reducing sugars and starch in the solution before and after the growth of the fungus; also the hydrolysis of starch by enzym powder*

[Expressed in milligrams per 10 cc. of solution]

Series.	Before inoculation.			After removal of fungus.			
	Total reducing sugars after digestion of starch.	Reducing sugars before digestion of starch.	Starch as reducing sugars.	Total reducing sugars after digestion of starch.	Reducing sugars before digestion of starch.	Starch as reducing sugars.	Hydrolysis of starch by enzym powder.
a.....	47.97	0	47.97	No starch left.	9.23	0	26.19
b.....	No starch used.	122.20	0	No starch used.	33.93	0	5.78
c.....	213.98	120.90	93.08	124.80	44.72	80.08	4.42
d.....	508.56	298.48	210.08	217.62	144.30	73.32	41.08

An examination of the foregoing results shows a clear case of the regulatory influence of the culture medium on the quantitative secretion of amylase. In every case where starch (series a) alone was used as the source of carbon the enzym powder hydrolyzed several times as much starch in a corresponding length of time as when glucose alone (series b) or in combination with starch (series c) was used. On the other hand, the enzym powder from sweet potato bouillon (series d), which always contained reducing sugars and starch and probably other carbohydrates, hydrolyzed considerably more starch than the powder from the a series. This exception is hard to explain, since it was obviously impossible to determine the exact composition of sweet potato bouillon. That it was a better medium for the growth of the fungus was quite evident. The quantity of felt was always greater than in any of the other series. The growth in the a series was likewise better than in either the b or c series, starch alone appearing to be a better source of carbon than glucose alone or in combination with starch.

These results seem to indicate that within the limits of these experiments the solution which is best for the growth of the fungus is likewise best for the secretion of amylase, regardless of the source of carbohydrates. It is probable that it is not so much the source of the carbohydrate which influences directly the quantitative production of the enzym as the influence it has upon the growth of the fungus on which the secretion of the enzym depends.

INFLUENCE OF THE AGE OF THE MYCELIUM WHEN REMOVED FROM THE CULTURE ON THE PRODUCTION OF AMYLASE

It was shown by Dox, Young, and others that the greatest amount of enzym is contained in the mycelium at about the beginning of the fruit-

ing period. So far as the writer is aware, this fact has not been determined for *Rhizopus tritici*, and it was with the view of verifying it for this fungus alone that comparative tests were made. In the experiments carried out by the writer two different culture media were used—namely, sweet potato bouillon and a modification of Czapek's nutrient solution with a 0.5 per cent starch paste as a source of carbon. In the former case the mycelium was removed from one set of flasks 3 days after inoculation, when fruiting was just beginning. The mycelium was removed from the other set of flasks 10 days after inoculation. The difference in reducing power in this case was not large.

On the other hand, when the Czapek's modified solution was employed, the mycelium removed 5 days after inoculation (when just beginning to fruit) hydrolyzed considerably more starch in a given length of time than the mycelium removed 10 days later.

SUMMARY

(1) A vigorous starch-splitting enzym is secreted by *Rhizopus tritici*. While some of the enzym is retained in the mycelium of the fungus, a portion of it diffuses out into the substratum. The diffusion into the culture medium begins soon after the substratum is inoculated, as was shown by some of the experiments in which reducing sugars appeared after 2 days in a nutrient solution with starch as the only source of carbon. The reducing sugars in such a medium accumulate in excess of the needs of the fungus.

(2) The enzym is able to act on raw sweet potato and Irish potato starch but much less energetically than on starch paste.

(3) The dried mycelium may be stored for several months at a temperature of from 9° to 35° C. without much deterioration, but at 60° it gradually becomes weaker.

(4) The optimum temperature for the digestion of starch is about 45° C. Above and below this temperature the amount of hydrolysis becomes less, and at 60° it is completely destroyed in 100 hours.

(5) If glucose is added to a system the hydrolysis of starch paste is retarded. The quantity of glucose added does not seem to influence the results. With a constant amount of enzym powder the total reducing sugars formed in a solution of starch paste increases with the increase in the volume of the solution up to a certain point and then decreases.

(6) An end point in the hydrolysis of the starch is not reached without altering the equilibrium of the system. This was done by changing the temperature and diluting the solution. If judged by the iodin test an end point was obtained, but a quantitative determination of the reducing sugars did not account for all the starch. It is probable that in this case some of the products of the hydrolysis were dextrins which were not accounted for as either starch or reducing sugars.

(7) When the enzym is in suspension some of it is removed by filtering through Whatman chemically prepared filter paper.

(8) The temperature at which the fungus is grown has a marked influence on the production of intercellular amylase. With an equal weight of enzym powder it was found that mycelium grown at 9° C. hydrolyzed about four times as much starch in the same length of time as mycelium grown at 40°. The enzym powder of mycelium grown at 29° was intermediate between the other two. At these three temperatures the best growth of the fungus was made at 29° and the poorest at 9°.

(9) The results of these investigations show that there is a "quantitative regulation" of the enzym. The hydrolyzing power of the mycelium grown on Czapek's modified nutrient solution was much greater when starch alone was used as a source of carbon than when glucose alone or in combination with starch was employed. On the other hand, if grown on sweet potato bouillon, which contains both starch and sugars, a unit weight of the mycelium will hydrolyze more starch than when grown on any of the other combinations. The vigor of growth of the fungus was correlated with the hydrolytic power of the enzym powder. The results seem to indicate that it is not so much the source of the carbohydrate which influences the quantitative production of the enzym as it is the influence which it has on the growth of the fungus on which the secretion of the enzym depends.

(10) The enzym powder of young mycelium just beginning to fruit was more active than the enzym from old mycelium.

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A COMPARATIVE STUDY OF THE COMPOSITION OF THE SUNFLOWER AND CORN PLANTS AT DIFFERENT STAGES OF GROWTH

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INTRODUCTION

The sunflower plant is gaining recognition as a silage crop in certain of the northwestern States where climatic or soil conditions are not always favorable for the maturing of corn for silage purposes. In some sections also there is a growing sentiment that sunflower silage offers a more profitable feed than corn silage, because of the greater yield that may be obtained per acre.

The Dairy Division is making an investigation of sunflower silage. This paper, which is the first of a series, presents the results of a study of the chemical composition of the sunflower plant at several different and distinct stages of its growth as compared with that of corn grown under similar conditions. The purpose of the study is to assist in selecting the proper stage of maturity for ensiling.

The investigation of the corn plant was made partly as a basis on which to study the sunflower plant and partly in connection with another investigation, the results of which will be published in a paper having to do with the fermentation of corn in the silo.

HISTORICAL REVIEW

Numerous analyses of the sunflower plant have been published from time to time. In some cases these have represented the whole plant, but more often only the head or the seed. No record of any study of the composition of the plant at different stages of growth has been found. On the other hand, there have been several such studies, more or less complete, made of the corn plant. Some of these will be briefly reviewed.

Roberts (*5*)[1] selected periods of growth (1) when the plants were coming into bloom, (2) when approaching roasting-ear condition, and (3) when most of the ears were out of the milk. Basing his figures on the dry matter, he found that the percentage of protein decreased from the first period to the last, while the percentage of carbohydrates increased.

Ladd (*3*) concludes that the nitrogen steadily diminishes throughout the period of growth, while the sugars rise and fall. The starch falls slightly during the earlier stages and then rises rapidly until the plant reaches maturity.

[1] Reference is made by number (italic) to "Literature cited," p. 792–793.

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Morse (*4*) analyzed samples representing four stages of growth and reached the same conclusions, with respect to the protein and carbohydrates, as the other investigators.

Perhaps the most elaborate study of the subject was made by Jones and Huston (*2*). Their study included the whole plant as well as the stalks, leaves, and ears taken separately. Unfortunately their figures for the whole plant are based upon yield per acre and so can not be compared with those of the other investigators or with ours.

EXPERIMENTAL WORK

The crops for the experimental work were grown in a section of the field at the Dairy Division Experiment Farm at Beltsville, Md., usually devoted to silage corn. The preparation of the soil, the planting, and cultivating were done under the supervision of T. E. Woodward, farm superintendent.

The sunflower plants were of the variety known as Giant Russian, and the corn was Boone County White. The sunflower plants thrived well in this soil (Bibb silt loam), reaching a height in many cases of 10 and 12 feet.

In dividing the growing period of the corn plant into stages, more or less arbitrary points must be taken. It is quite useless for the purpose to select plants by their age or height, for it is easily possible to find at any one time within a comparatively small area plants of the same height and age at entirely different stages of maturity. Up to the time of tasseling, however, there are no easily recognized guides except height. From that time until the plant is fully mature there are certain and fairly distinct points that can be selected, based on the condition of the silk and ears.

The task of selecting stages of growth of the sunflower plant offers more difficulty, and it is quite impossible to divide it into anything like as sharply defined stages as in the case of the corn plant. We endeavored to differentiate the stages first by the height and later by the condition of the flower and seed, but at best these points are very arbitrary.

The difficulties in selecting representative samples of whole plants for chemical analysis are obvious. The plan we followed was to go through a small area of the field and select from 6 to 20 plants of the proper stage of growth and as nearly the same size and conformity as possible. These were carefully wrapped in a specially prepared waterproof cloth and taken immediately to the laboratory, where they were cut into 1-inch lengths with a hand-power feed cutter.

A 1-kilogram subsample was weighed out and dried in the steam closet for the determination of starch. The remainder was ground to a pulp in a power meat grinder, and a subsample was taken for moisture, albuminoids, and total-protein determinations. A further subsample

was weighed out, from which the alcoholic extract of the pulp was prepared according to the method described by Swanson and Tague (*6*). Aliquot portions of the alcoholic extract were used to determine total and reducing sugars according to the gravimetric cuprous-oxid method of Walker and Munsen (*7, p. 241*).

Moisture was determined on a 5-gm. sample of the pulp by drying to constant weight in a reduced pressure water-jacketed oven. The subsample dried in the steam closet was ground to pass a 40-mesh sieve, and starch was determined on the air-dry sample by the diastase method with subsequent acid hydrolysis (*1, p. 110*).

Tables I and II give the results of the chemical work on the whole plants. The figures for total protein, albuminoid protein, reducing sugars, nonreducing sugars, and starch are based on the dry matter.

TABLE I.—*Composition of sunflower plant at different stages of growth*

Stage of maturity.	Moisture in fresh material.	Dry matter.	Moisture-free basis.				
			Total protein.	Albuminoid protein.	Reducing sugars.	Nonreducing sugars.	Starch.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
3 feet high	84.87	15.13	8.59	8.00	12.36	19.08	0.63
6 feet high	86.02	13.98	8.01	7.37	18.95	15.63	4.61
First flower	84.09	15.91	7.04	6.35	15.96	8.43	4.34
Rays ready to fall	83.90	16.10	9.44	7.89	13.23	3.01	.20
Rays dry and partly fallen	75.58	24.42	6.80	6.22	8.96	1.40	.84
Rays all fallen	74.37	25.63	7.03	6.09	6.99	.89	1.66
Seeds hard and mature	69.68	30.32	5.90	5.04	4.15	1.47	1.90

TABLE II.—*Composition of corn plant at different stages of growth*

Stage of maturity.	Moisture in fresh material.	Dry matter.	Moisture-free basis.				
			Total protein.	Albuminoid protein.	Reducing sugars.	Nonreducing sugars.	Starch.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
3 feet high	84.21	15.79	11.14	10.26	14.69	2.73	1.52
4½ to 5 feet high	85.14	14.86	9.42	8.14	16.69	3.23	1.66
Just tasseling	81.65	18.35	9.90	6.59	13.13	1.85	1.29
Just silking	81.56	18.44	8.95	6.73	18.23	1.30	.86
Kernels forming	81.20	18.80	8.99	6.38	20.37	5.44	3.45
Milk stage	77.60	22.40	8.97	6.30	17.59	4.51	2.87
Silage stage (one-half milk, one-half glazed)	68.69	31.31	7.31	6.23	10.03	2.81	24.00
All glazed	64.22	35.78	6.32	5.62	8.50	5.39	24.78
Ready to shock	59.79	40.21	7.09	6.14	7.71	2.73	21.66

DISCUSSION OF RESULTS

In studying the tables it should be borne in mind that the figures represent percentages based on the plants themselves and have no bearing on the yield of the various constituents per unit of area. For example, the proteids decline in percentage as the plant grows. This does not mean, of course, that the amount of the proteids per given area decreases, but rather that as the plant grows and increases in weight the proteids do not increase in the same ratio.

Too much importance must not be placed on slight differences in composition from stage to stage of growth. Because of the difficulties in sampling whole plants, small differences due to unavoidable errors are to be expected, and conclusions are safest when drawn from the general trend of the results rather than from particular figures.

Considering the sunflower plant first, it will be noted that the dry matter steadily increases as the plant grows older. This, of course, is what would be expected, but the fact is rather surprising that, even after the rays had all fallen and the seeds had become dry and mature, the plant still contained more moisture than the corn contained at the time it was ready for the silo.

The proteids, both total and albuminoid, show a tendency to decline as growth proceeds. This is somewhat contrary to what might be expected from the highly nitrogenous character of the seed.

The reducing sugars rise and then gradually decline. The nonreducing sugars steadily and rapidly decline throughout the whole period of growth. In the first stage there is one and one-half times as great a quantity of nonreducing sugars present as reducing sugars. This relation, however, is quickly changed, and in the last stage there is nearly three times as much of reducing sugars present as nonreducing. The percentage of starch is small, rising and falling with no apparent relation to the change in percentage of the sugars.

Turning now to the corn plant, it will be noted, as would be expected, that the dry matter steadily increases as the plant grows older. The proteids, both total and albuminoid, decline slowly but quite regularly. The sugars, both reducing and nonreducing, rise and fall but have an upward trend until the kernels begin to mature, when there is a sharp drop, accompanied by a sudden increase in the starch. This is at the stage when the plant is storing starch in the kernels and is the stage usually selected for ensiling. The ratio of reducing and nonreducing sugars changes, but within a somewhat narrow range. The reducing sugars always greatly exceed the nonreducing. The starch rises and falls up to the stage when the kernels begin to mature. Between the milk stage and what may be called the silage stage the starch increased from 2.87 per cent to 24 per cent.

Comparing the sunflower and the corn plants, it will be noted that the chief difference in the constituents studied lies in the amount and char-

acter of the carbohydrates. Although no part of the present experiment, silage was made of the sunflower plant at different stages of maturity, and it was found that silage made from plants at the stage when the rays were dry and partly fallen was excellent in quality. Comparing the plant at this stage with the corn plant at the silage stage, it will be seen that the starch and sugars combined constitute 11.2 per cent of the dry matter in the former, of which only about one-fifteenth is starch, while the combined starch and sugars in the dry matter of the latter constitute nearly 37 per cent, two-thirds of which is starch.

There is no great difference in the percentage of proteids in the dry matter of the two plants, but it is slightly in favor of the corn plant.

In selecting the best stage of maturity of a plant for ensiling, several things must be taken into consideration. In general the stage must be selected that promises the largest yield of food constituents in the silage. This stage is not necessarily the one when the plant itself has the maximum amount of food constituents. The moisture content of a plant, judging by the behavior of the corn plant when ensiled, plays an exceedingly important rôle. When silage is made from the corn plant having a high moisture content there is a downward seepage of the juice, carrying with it valuable food material. If the silo is tight this juice waterlogs the bottom layer, rendering it unfit for feeding. If the silo is not tight the juice leaks out and is lost altogether. Moreover, high moisture in the plant is usually associated with high-acid silage. On the other hand, a plant that has too low a moisture content is difficult to pack closely enough to eliminate the air spaces that cause spoilage. Silage produced from such plants is dry and lacks palatability.

Another point that should not be lost sight of is, of course, the yield per acre. This point, aside from the high moisture content, would bar out the three earlier stages of the sunflower plant. The fourth stage is still too high in moisture. The last stage contains nearly 70 per cent of moisture.

From the moisture content alone the sunflower plant at this stage should make good silage, but here another factor must be taken into consideration. The sunflower plant at this stage has lost some of its leaves. The outer part of the stalk has become so hard and woody that it would be difficult, if not impossible, to pack it closely enough to prevent spoilage. This eliminates all but two stages, the one when the rays are dry and partly fallen and the other when all the rays have fallen. These stages are close together, and judging from the chemical composition there is but little choice between the two.

There is but little difference in percentage between the total proteids and albuminoid proteids in the sunflower plant at these stages and the corn plant at the silage stage. The chief differences, as discussed in another paragraph, lie in the sugars and starch.

SUMMARY AND CONCLUSIONS

A study was made of the chemical composition of the sunflower and corn plants at different stages of growth.

The dry matter in each increased gradually and consistently throughout the entire period of growth.

There is no great difference in the percentage of proteids in the two plants, but it is slightly in favor of the corn plant.

The reducing and nonreducing sugars in the sunflower declined somewhat irregularly but persistently during the growth of the plant. In the first stage there was about one and one-half times as much nonreducing sugars present as reducing sugars. This relation was quickly changed, and in the latter stages the reducing sugars greatly exceeded the nonreducing.

The percentage of starch in the sunflower is small and rises and falls irregularly throughout the growth of the plant.

The reducing and nonreducing sugars in the corn plant rise and fall but with a marked upward trend during the growth of the plant until the stage is reached where the kernels are maturing, when a sudden drop occurs. The percentage of reducing sugars is always far in excess of the nonreducing sugars.

The starch rises and falls until the kernels are maturing, when a sudden rise occurs.

The chief difference between the two plants at the silage stage lies in the amount and character of the carbohydrates.

From the results obtained in this study it would seem that the best stage of maturity for ensiling the sunflower plant is when the rays of the flower have become dry and are falling.

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